Disorders of platelet number and function

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The online version of this chapter contains an educational multimedia component on platelet function in health and disease.

Conflict-of-interest disclosure: Hanny Al-Samkari: consultancy: Agios, Dova, Argenz, Sobi, Rigel; research funding: Agios, Amgen, Dova. Michele P. Lambert: consultancy: Amgen, Novartis, Shionogi, Dova, Principia, Argenx, Rigel, Bayer; research funding: Sysmex, Novartis, Rigel, Astra Zeneca; advisory board: Octapharma, Shionogi.

Off-label drug use: Desmopressin for inherited platelet function defects and uremic platelets. Recombinant VIIa for inherited platelet function defects. Rituximab for ITP and TTP. Fondaparinux, bivalirudin, and direct oral anticoagulants for HIT.

Platelet biology: structure and function

Hemostasis encompasses a series of interrelated and simultaneously occurring events involving the blood vessels, platelets, coagulation system, and the fibrinolytic pathway. Defects affecting any of these major participants may lead to a hemostatic defect and a bleeding disorder. This chapter focuses on disorders related to platelet number and function.

Platelet structure

Blood platelets are anucleate fragments derived from bone marrow megakaryocytes. The platelet diameter ranges from 1.5 to 3.0 μm, roughly one third to one fourth that of an erythrocyte. Mean platelet volume is approximately 7-10 fL. Electron microscopy reveals a fuzzy coat (glycocalyx) on the platelet surface composed of membrane glycoproteins (GPs), glycolipids, mucopolysaccharides, and adsorbed plasma proteins. The plasma membrane is a bilayer of phospholipids in which cholesterol, glycolipids, and GPs are embedded. The phospholipids are asymmetrically organized in the plasma membrane; negatively charged phospholipids (such as phosphatidylserine [PS]) are present almost exclusively in the inner leaflet, whereas the others are more evenly distributed. Platelets have an elaborate channel system, the open canalicular system, which is composed of invaginations of the plasma membrane and extends throughout the platelet and opens to the surface. The discoid shape of the resting platelet is maintained by a well-defined cytoskeleton consisting of the spectrin membrane skeleton, the marginal microtubule coil, and the actin cytoskeleton. The microtubule coil, present below the platelet membrane, is made up of α-β-tubulin dimers and, together with nonmuscle myosin IIA, plays a role in platelet formation from megakaryocytes, in addition to maintaining the discoid platelet shape. In proximity to the open canalicular system is the dense tubular system, a closed-channel network derived from the smooth endoplasmic reticulum. It is considered the major site of platelet prostaglandin and thromboxane synthesis.

Platelets contain a variety of organelles: mitochondria and glycogen stores, lysosomes, peroxisomes, dense granules, and α-granules. The lysosomes contain acid hydrolases; the dense granules contain calcium (which gives them high electron density), adenosine triphosphate (ATP), adenosine diphosphate (ADP), magnesium, serotonin (5-hydroxytryptamine), and polyphosphates (which

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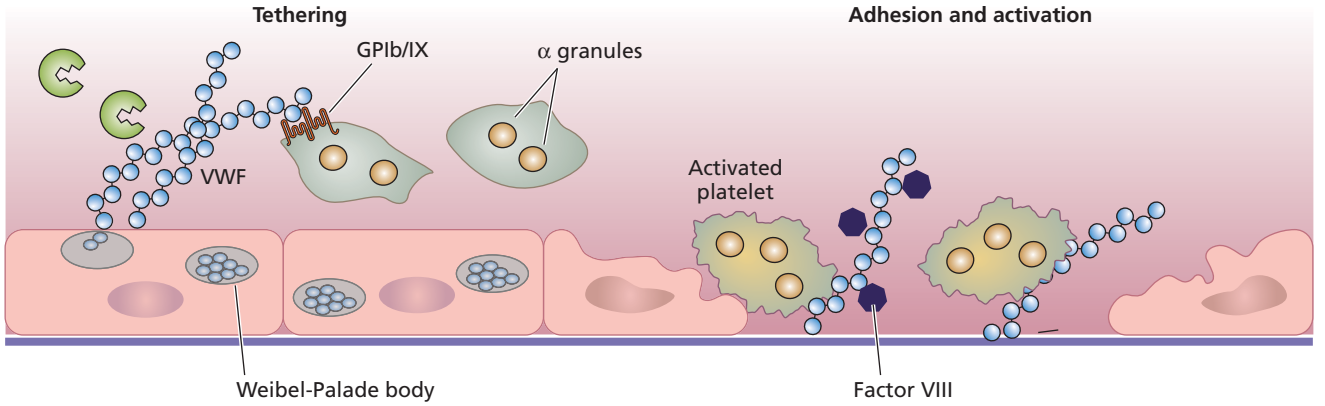
promote coagulation through various means, including activation of the intrinsic pathway). Serotonin is taken up by platelets from plasma and incorporated into the dense granules. The α-granules contain a large number of proteins, including β-thromboglobulin (βTG) and platelet factor 4 (PF4), which are considered platelet specific; several coagulation factors (eg, fibrinogen, factor V, and factor XIII); von Willebrand factor (VWF); growth factors (eg, platelet-derived growth factor and vascular endothelial growth factor); vitronectin; fibronectin; thrombospondin; the factor V binding protein: multimerin; P-selectin; albumin; and immunoglobulin G (IgG). Some of these (eg, VWF, PF4, and βTG) are synthesized by megakaryocytes, whereas others (eg, albumin and IgG) are incorporated into the α-granules from plasma.

Platelet function in hemostasis

Following injury to the blood vessel (see video in online edition), platelets interact with subendothelial and plasma proteins to roll along (tether) and eventually stop (adhesion) at sites of vascular injury (Figure 11-1). Platelet function can be broken into 2 main steps with key modulators of each step (adhesion and amplification). In platelet adhesion, the major contributor under conditions of high shear stress is the interaction of plasma VWF with its platelet receptor, GP Ib-IX-V. After adherence to the vessel wall via VWF and the long GP Ib-IX-V receptor, other platelet receptors interact with proteins in the subendothelial matrix, allowing for the interaction of GPVI (an important immunoreceptor-typrosine-based activation motif [ITAM]-containing receptor) with collagen and GPIIb/IIIa (intergrin αIIbβ3) with fibrinogen. These interactions provide not only a surface for adhesion but also serve as a strong downstream signal for platelet activation signals through Src kinases and through effector recruitment and substrate phosphorylation, eventually causing activation of phospholipase Cγ2. Activated platelets release the contents of their granules (secretion), including ADP, serotonin (5 hydroxytryptamine: 5HT), and thromboxane A_2 (TXA_2), as well as thrombin/thrombin-like peptides. These soluble mediators then amplify platelet activation by activating G protein–coupled receptors (GPCRs) that act as molecular switches (primarily the purinergic receptors $P2Y_1$ and $P2Y_{12}$, Thromboxane A_2 Receptor, and the protease-activated receptors PAR1 and PAR4). This amplification signal now initiates phospholipase Cβ activation to cause the conversion of phophoinositide-4.5-bisphosphate (PIP2) to inositol-1,4.5-triphosphate (IP3) and 1,2-diacyl-glycerol (DAG). IP3 causes the release of intracellular Ca^{2+}, and DAG activates protein kinases C (PKCs), which cause sustained platelet granule secretion, further signal amplification, and GPIIb/IIIa receptor activation (Figure 11-2). Ca^{2+} and PKC both can activate GPIIb/IIIa separately but act synergistically to induce a conformational change that leads to fibrinogen binding. Ca^{2+} acts rapidly through CalDAG-GEF1, a guanine nucleotide exchange factor that actives Rap1b (a GTPase) to quickly provide "inside-out" activation of GPIIb/IIIa. PKC acts independently of CalDAG-GEF1 to provide more sustained activation of Rap1. Both induce cytoskeletal rearrangements, platelet granule extrusion, and conversion of GPIIb/IIIa to the high-affinity state. Subsequent ligand interactions (mainly fibrinogen) with GPIIb/IIIa bridges platelets together, triggering "outside-in" signaling. Moreover, platelets play a major role in coagulation mechanisms. Several key enzymatic reactions occur on the platelet membrane lipoprotein surface. During platelet activation, the negatively charged phospholipids, especially PS, become exposed on the platelet surface, an essential step for accelerating specific coagulation reactions by

Figure 11-1 Schematic representation of platelet responses starting with tethering, then platelet adhesion of GPIb-IX to VWF, and then platelet activation.



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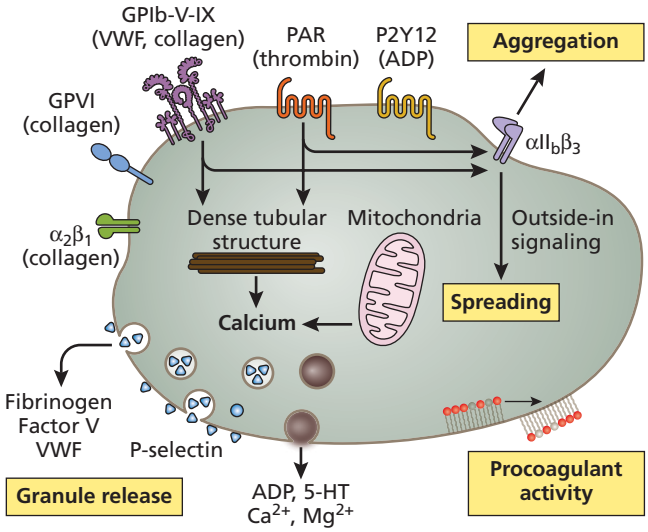

Figure 11-2 Amplification and signal transduction in platelet responses. Platelets can undergo activation through stimulation by soluble agonists, such as thrombin, or by contact (adherence) to the subendothelial matrix. This simplified cartoon shows several platelet components, including receptors and granules, as well as the pathways of activation and the effect on platelet responses, such as aggregation, spreading, granule release, and procoagulant activity.

promoting the binding of coagulation factors involved in thrombin generation (platelet procoagulant activity).

Either inherited or acquired defects in these platelet mechanisms may lead to impairment of platelet function in hemostasis.

KEY POINTS

- Platelets are small, anucleate cells with a mean platelet volume of ~7-10 fL.
- The 3 main types of granules within platelets are lysosomes (containing acid hydrolases), dense granules (dense because of calcium and containing also ADP, ATP, and serotonin), and alpha granules (containing proteins synthesized and taken up by platelets such as PF4, VWF, and IgG).
- Platelet adhesion begins with platelet binding to subendothelial collagen and platelet interactions between GP1b/IX/V and plasma VWF.
- Release of soluble modulators (ADP, 5HT, and TXA_2) amplify signals to enhance platelet activation. Further platelet activation through intracellular calcium mobilization, protein phosphorylation, and tyrosine kinase activation results in inside-out activation of GPIIb/IIIa and completes activation.
- Inherited or acquired defects in these mechanisms can impair hemostasis.

Platelet function testing

PFA-100

The PFA-100 (platelet function analyzer) is a widely available laboratory test that may be abnormal in some congenital and acquired platelet function disorders. The PFA-100 induces high shear stress and simulates primary hemostasis by flowing whole blood through an aperture with a membrane coated with collagen and either adenosine diphosphate (ADP) or epinephrine. Platelets adhere to the collagen-coated surface and aggregate, forming a platelet plug that enlarges until it occludes the aperture, causing cessation of blood flow. The time to cessation of flow is recorded as closure time (CT). It may be useful to screen individuals with suspected platelet dysfunction or von Willebrand disease (VWD), although it has a number of limitations, including limited sensitivity in patients with mild-to-moderate platelet function disorders or VWD. The PFA-100 is described in greater detail in Chapter 12.

Platelet aggregometry

The most specific assay of platelet function is platelet aggregation by light transmission aggregometry, which remains the gold standard in platelet function testing. This assay uses platelet-rich plasma and evaluates platelet aggregation via light transmission after the addition of a variety of agonists, such as ADP, epinephrine, ristocetin, arachidonic acid, collagen, and thrombin-related activation peptide. Patients with a variety of both severe and mild platelet function disorders exhibit abnormal platelet aggregation profiles, and furthermore, the spectrum of abnormalities can be diagnostic of specific disorders. Platelet aggregometry is described in greater detail in Chapter 12.

Flow cytometry

Flow cytometry may be employed to quantify levels of platelet surface receptors and can confirm the diagnosis of Bernard-Soulier syndrome (BSS) and Glanzmann thrombasthenia. In some institutions, these assays are available and have become the method of choice for diagnosis but have not been standardized for widespread use.

Electron microscopy

Some platelet function defects lead to easily identifiable platelet ultrastructural changes visualized by electron microscopy. In particular, patients with a deficiency or abnormalities of dense bodies (δ-storage pool deficiency) or α-granules (gray platelet syndrome [GPS] and Paris-Trousseau syndrome) can be diagnosed by this method, particularly since dense granule deficiency may not be associated with obvious defects on light transmission aggregometry.

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KEY POINTS

- Screening tests for platelet disorders, such as the PFA-100, have limited value. The gold standard laboratory evaluation for platelet function disorder involves platelet aggregation studies, but these have limitations as well. These studies and their limitations are described In greater detail in Chapter 12.
- Flow cytometric methods to assess platelet function have been developed but are currently used primarily on a research basis, with some exceptions.
- Electron microscopy is an important modality in the identification of platelet storage pool deficiencies.

Regulation of platelet number

Overview

The platelet count is regulated by the relative rates of platelet production and clearance. Kinetic studies have demonstrated that the average platelet life span is 7 to 10 days. Platelets that are lost through senescence, activation, or other processes are replaced by new platelets derived from bone marrow megakaryocytes. Platelet production from megakaryocytes, in turn, is driven by the hormone thrombopoietin (TPO) and its cellular receptor, c-Mpl.

Thrombopoietin and the thrombopoietin receptor c-Mpl

A healthy adult produces 1×10^{11} to 3×10^{11} platelets per day, although production can increase 10-fold during times of high demand. The number of circulating platelets is regulated chiefly by TPO, which binds to megakaryocytes and hematopoietic stem cells via c-Mpl, which is a member of the class I hematopoietic growth factor receptor superfamily and activates several signaling pathways in megakaryocytes, resulting in megakaryocyte proliferation and differentiation, ultimately resulting in platelet production. c-Mpl is also expressed on mature platelets, which bind and clear TPO from the circulation. TPO is secreted constitutively from the liver; although its synthesis may increase slightly during thrombocytopenic states, its overall production is relatively constant. As a consequence, the level of free TPO is regulated primarily by the number of circulating platelets, the platelet life span, and the megakaryocyte mass. Recent mouse studies have challenged this paradigm, however. The Ashwell-Morell receptor on murine hepatocytes binds platelets that have lost sialic acid residues on their surface. Binding activates a JAK-STAT signaling pathway, resulting in increased hepatic TPO mRNA and TPO production. The relevance of this pathway to normal human thrombopoiesis is not yet known. Additional pathways regulating platelet production are also important, evidenced by profound thrombocytopenia in animals and humans lacking TPO signaling but not absent megakaryopoiesis, as well as a known role of inflammatory cytokines in regulating TPO mRNA expression, particularly interleukin (IL) 6.

In conditions such as aplastic anemia, which is characterized by a low platelet count and decreased bone marrow megakaryocyte mass, free TPO levels are very high. In immune thrombocytopenia (ITP), the megakaryocyte mass may be expanded, and platelet clearance is accelerated. This results in enhanced TPO clearance and normal or only slightly elevated plasma TPO levels, despite thrombocytopenia. The role of TPO as the principal physiologic regulator of platelet production has been confirmed in studies of TPO- and c-Mpl-deficient mice, which have 5% to 15% of normal levels of circulating platelets, megakaryocytes, and megakaryocyte progenitor cells. TPO alone, however, does not fully support megakaryocyte polyploidization in vitro, suggesting that additional factors—such as stem cell factor, interleukin 3, interleukin 6, and interleukin 11—are required for optimal megakaryocyte development.

Normal platelet production

Megakaryocyte proliferation and differentiation involve endomitosis and polyploidization, a process in which the nucleus divides but the cell does not. In the process of maturation, megakaryocytes form secretory granules and a demarcation membrane system that permeates the cytoplasmic space. This extensive membrane system eventually projects multiple filamentous pseudopodial structures called proplatelets. This process utilizes the entire repertoire of cytoplasmic granules, macromolecules, and membranes. Ultimately, fragmentation of the pseudopodial projections leads to the release of new platelets. The exact steps leading from megakaryocytes to mature platelets are still not fully resolved. Different mechanisms are proposed and have been shown in mouse models: (1) at the sinusoids of the bone marrow, the megakaryocytes produce long proplatelet strings, from which individual platelets rupture; (2) larger fragments of megakaryocytes consisting of proplatelets are released into the sinusoids, which then divide into individual platelets in the circulation; and (3) the entire megakaryocyte migrates into the sinusoids and is then transported in the bloodstream into the lung, where the shear forces in the lung arterioles cause release of platelets. It is likely that all 3 models are at least partially true and together contribute to platelet production from megakaryocytes. Each megakaryocyte

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produces 1000 to 3000 platelets before the remaining nuclear material is phagocytosed by resident macrophages. Released platelets circulate for 7 to 10 days before undergoing senescence and clearance by phagocytic cells in the reticuloendothelial system.

KEY POINTS

- The primary mediator of platelet production is TPO, produced primarily by the liver.
- TPO production is largely constitutive; TPO levels are regulated by the platelet and megakaryocyte mass through binding of TPO to its receptor, c-Mpl.
- TPO levels are typically normal in ITP, representing relative deficiency compared to platelet count, but are elevated in bone marrow failure syndromes.
- The normal platelet life span is 7 to 10 days.

Immune thrombocytopenia

ITP is an autoimmune disorder characterized by thrombocytopenia and a variable risk of bleeding. An international working group proposed standard terminology and definitions for ITP. The term *immune* is now used instead of *idiopathic*, and the term *purpura* has been abandoned because bleeding symptoms, including purpura, are not present in all cases. Thus, the working group recommended the term *immune thrombocytopenia*, although the abbreviation ITP is preserved. In the working group's classification scheme, *primary* is used to denote ITP with no apparent precipitating cause, whereas *secondary ITP* refers to immune-mediated thrombocytopenia in which a predisposing condition can be identified. ITP is also classified according to disease duration. Within 3 months of presentation, ITP is termed newly diagnosed. ITP lasting 3 to 12 months and >12 months is denoted as *persistent* and *chronic*, respectively (Table 11-1). This terminology was adopted in the ITP guideline developed by the American Society of Hematology (ASH).

Although it is a rare disease, ITP is a relatively common cause of thrombocytopenia in adults and children. Estimates of prevalence vary, ranging between 3 and 20 per 100,000 persons, with an estimated incidence of 2 to 10 cases per 100,000 patient years. In childhood, the highest incidence is in children <5 years old, with a gradual decrease toward adolescence. Most studies find the incidence to be equal in girls and boys, although some reports suggest a higher incidence in boys <5 years old. In adults, the incidence and prevalence of ITP is greatest in the elderly, with a female preponderance in the middle-adult years and a slight male preponderance in patients >70 years of age. In children, ITP often occurs after an antecedent viral infection and is self-limited in 80% of cases. In contrast, primary ITP assumes a chronic course in approximately 75% of adult patients. Primary ITP may also occur following receipt of vaccines. Although patients with more severe thrombocytopenia may present with mucocutaneous bleeding, those diagnosed with thrombocytopenia on a routine blood count are often asymptomatic. There is no gold-standard laboratory test for ITP. Although detection of glycoprotein-specific antiplatelet antibodies on the patient's platelets suggests the diagnosis, sensitivity of antiplatelet antibodies has been more variable (with a recent meta-analysis suggesting a sensitivity of 50%-60%). Moreover, antiplatelet antibodies may be detected in thrombocytopenic patients without ITP (eg, in microangiopathies in which damaged platelets expose immunogenic epitopes). The diagnosis of ITP is primarily made by excluding other causes of thrombocytopenia and investigating potential secondary causes. The most compelling evidence supporting a diagnosis of ITP is a platelet response to ITP-specific therapy. Consideration of alternative causes of thrombocytopenia, particularly in a patient who has failed to respond to typical ITP therapy and prior to splenectomy, prevents misdiagnosis of underlying congenital thrombocytopenia in up to 20% of adults with initial diagnosis of ITP.

Secondary ITP occurs in the setting of lymphoproliferative disorders; systemic lupus erythematosus, antiphospholipid syndrome, or other autoimmune disorders; infections such as hepatitis C (HCV), HIV, and *Helicobacter pylori*; and immune deficiency states such as common variable immune deficiency. Drug-induced

Table 11-1 ITP definitions

Primary ITP	• Isolated thrombocytopenia • Platelets $<100 \times 10^9$/L • No other apparent causes of thrombocytopenia • No secondary cause of ITP present
Secondary ITP	• All other forms of immune-mediated thrombocytopenia except primary ITP • Designate with presumed cause, in parentheses (eg, lupus associated)
Phases of ITP	• Newly diagnosed: within 3 months of diagnosis • Persistent: between 3 and 12 months of diagnosis • Chronic: lasting >12 months

Adapted from Rodeghiero F et al, *Blood*. 2009;113:2386-2393.

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immune thrombocytopenia (DITP) is described in the section "Drug-induced immune thrombocytopenia" later in this chapter. Nonimmune causes of thrombocytopenia, including hypersplenism, hereditary thrombocytopenias, thrombotic thrombocytopenic purpura (TTP), and type 2B von Willebrand disease, should be included in the differential diagnosis of ITP. Occasional patients with myelodysplastic syndromes or aplastic anemia may present with isolated thrombocytopenia.

Clinical features of ITP

Clinical features of primary and secondary ITP are generally similar, although in secondary ITP, clinical manifestations related to the underlying disorder may be prominent. A platelet count below 100×10^9/L is required for the diagnosis of ITP because mild thrombocytopenia may occur in normal individuals and uncommonly results in development of more severe thrombocytopenia or other autoimmune disease. The most common symptom of ITP is mucocutaneous bleeding, which may manifest as petechiae, ecchymoses, epistaxis, menorrhagia, oral mucosal, or gastrointestinal bleeding. In a systematic review, intracranial hemorrhage was reported in 1.4% of adults and 0.4% of children.

Spontaneous bleeding is uncommon at platelet counts $>30 \times 10^9$/L. There is significant variability in bleeding among patients with similar platelet counts, however, and some individuals with counts $<10 \times 10^9$/L bleed infrequently. The risk of fatal bleeding is greatest in elderly patients with persistent and severe thrombocytopenia ($<20 \times 10^9$/L) or in those patients with additional risk factors for hemorrhage, including need for antiplatelet or anticoagulation therapies. Nonhemorrhagic clinical manifestations common among patients with ITP include fatigue and reduced health-related quality of life. Fatigue may track with platelet count in certain patients and may improve with platelet-raising therapy in some of these patients. Mounting epidemiologic evidence suggests that ITP is associated with an increased risk of venous thromboembolism. The mechanism of thrombosis is not well established but may relate to underlying disease pathophysiology and/or treatment effect.

Physical examination should focus on typical bleeding sites. Dependent areas and skin underneath tight clothing should be examined for petechiae and purpura, and oral mucous membranes should be examined for hemorrhagic bullae, which may be associated with an increased risk of severe bleeding at other sites. In a patient with primary ITP, the remainder of the general physical examination is normal. The presence of lymphadenopathy or splenomegaly should prompt investigation for other etiologies of thrombocytopenia. Skeletal, renal, or neurologic abnormalities suggest a familial cause of thrombocytopenia.

Pathophysiology of ITP

Primary ITP is a syndrome that results from several different pathophysiologic mechanisms. Classic experiments performed in the 1950s and 1960s demonstrated a critical role for antiplatelet antibodies in mediating the enhanced clearance of platelets in patients with ITP. These antibodies recognize GPs on the platelet surface, most commonly GPIIb-IIIa and GPIb-IX. Antibody-coated platelets are cleared from the circulation by phagocytes in the reticuloendothelial system, primarily the spleen. Antiplatelet antibodies may recognize the same targets on megakaryocytes, leading to impairment of megakaryocyte proliferation and differentiation and proplatelet production. As noted previously, plasma levels of TPO generally are not elevated in patients with ITP because of an expanded megakaryocyte mass and accelerated platelet clearance. Not all patients with ITP have detectable antiplatelet antibodies. Dysregulated T cells may have a direct cytotoxic effect on platelets and impair platelet production by megakaryocytes. Recent interest has focused on decreased levels of regulatory T cells in patients with ITP; successful ITP treatment has been associated with restoration of regulatory T cell levels, although this has not been shown to lead to long-term remission of the underlying ITP.

The pathogenesis of secondary ITP may share similar mechanisms with primary ITP, although unique mechanisms have been identified in some types of secondary ITP. For example, antigen mimicry, in which antibodies directed to a foreign (viral) protein cross-react with specific epitopes on platelet GPIIb-IIIa, has been observed in hepatitis C–associated ITP. A similar pathophysiology may underlie the pathogenesis of ITP in patients with *H. pylori* infection and HIV.

Diagnosis of ITP

The diagnosis of ITP rests on a consistent clinical history, physical examination, and exclusion of other causes of thrombocytopenia. The leukocyte count is characteristically normal. The hemoglobin concentration is typically normal as well, unless thrombocytopenic bleeding has resulted in anemia. Examination of the peripheral blood film should be performed to exclude pseudothrombocytopenia (ethylenediaminetetraacetic acid-dependent platelet agglutinating antibodies), microangiopathic hemolytic anemia (MAHA; fragmented red cells), or abnormalities suggestive of other disorders. Identification of unexpected abnormalities should prompt an evaluation for other etiologies of thrombocytopenia.

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The mean platelet volume may be increased in patients with ITP. However, ITP patients always show a heterogeneous platelet population with not more than ~30% enlarged platelets, and historical platelet counts (if available) will be unremarkable with a normal mean platelet volume. If the blood smear shows more than 60% large or even giant platelets, hereditary macrothrombocytopenia (see "Hereditary thrombocytopenia" in this chapter) is more likely.

Bone marrow examination is not required routinely and is generally not useful for diagnosing ITP but should be performed to exclude other causes of thrombocytopenia when atypical features, such as unexplained anemia, lymphadenopathy, or splenomegaly, are present. Because at least 80% of patients with ITP respond to initial therapy with corticosteroids, intravenous immunoglobulin (IVIG), or Rh-immune globulin (anti-D), failure to respond to these agents should prompt consideration of bone marrow examination and investigation into other potential causes of thrombocytopenia. Bone marrow examination may also be warranted in elderly patients in whom myelodysplasia is suspected. Megakaryocyte number is typically normal or increased in the marrow of patients with ITP. In an anonymized study, hematopathologists were not able to reliably distinguish ITP marrows from those of nonthrombocytopenic controls.

With appreciation that secondary causes of ITP may be more common than previously believed and may influence management, additional laboratory studies, such as screening for hepatitis C and HIV, should be considered in adult patients presenting with suspected ITP. Table 11-2 contains a list of suggested screening studies proposed by the ITP International Working Group. A recent International Working Group consensus document reported that the majority of practitioners primarily treating adult ITP patients routinely screen for hepatitis B, HIV, and hepatitis C in newly diagnosed patients, and the most recent American Society of Hematology ITP guidelines recommend testing for HCV and HIV. Additionally, in patients who will be treated with intravenous immunoglobulin, it is reasonable to obtain quantitative immunoglobulin (Ig) levels prior to treatment to exclude the presence of underlying immune deficiency and, in some patients, malignancy. In pediatric patients with chronic ITP, periodic reevaluation of Ig levels is important to evaluate for an underlying cause of ITP.

Management of primary ITP in children

Because spontaneous recovery is expected in most children with primary ITP, families of children generally need counseling and supportive care rather than specific drug therapy. Severe hemorrhage occurs in ~1 in 200 children with newly diagnosed ITP, and intracerebral hemorrhage occurs in ~1 in 500 (based on data from the International ITP Cooperative Study Group). For those in whom treatment is considered necessary, a short course of corticosteroids, IVIG, or anti-RhD immune globulin (anti-D) in Rh-positive individuals generally results in rapid recovery of the platelet count and is not associated with differences in long-term development of chronic ITP. Adverse effects of therapy in children include behavioral changes from corticosteroids, headache/allergic reaction from IVIG, and hemolysis from anti-D, which rarely may be severe. Patients (adults and children) with a positive direct antiglobulin test should not receive anti-D because of an increased risk of severe hemolysis.

Table 11-2 International Working Group recommendations for the diagnosis of ITP in adults

Basic evaluation	Tests of potential utility	Tests of uncertain benefit
Patient and family history	Glycoprotein-specific antibodies	TPO levels
Physical examination	Antiphospholipid antibodies	Reticulated platelets/immature platelet fraction
CBC and reticulocyte count	Antithyroid antibodies and thyroid function	
Peripheral blood film	Pregnancy test in women of childbearing potential	
HBV	PCR for parvovirus, EBV and CMV	Bleeding time
Blood group (Rh)	Bone marrow exam	Complement levels
HIV, HCV (suggested by majority regardless of geographic region)	*H. pylori*	
Quantitative immunoglobulins (consider in children with ITP, recommend in children with persistent or chronic ITP)	Direct antiglobulin test	

Adapted from Provan D et al, *Blood Adv*. 2019;3(22):3780-3817.

CBC, complete blood count; CMV, cytomegalovirus; HBV, hepatitis B virus; PCR, polymerase chain reaction.

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Recovery of the platelet count ultimately occurs in ~80% of children even without therapy, usually within 3 to 6 months but occasionally over a year or more after presentation. Even in children with chronic ITP, major bleeding is uncommon, and thresholds for treatment are based more on bleeding symptoms and other concerns (fatigue and impact on quality of life) than on specific platelet counts. While a platelet count of <10,000 is permissive for bleeding, the vast majority of children with platelet counts even below this threshold do not require pharmaceutical or surgical treatment and can be safely monitored until recovery. Current American Society of Hematology guidelines recommend the use of short courses of corticosteroids and outpatient management of thrombocytopenia over IVIG in patients who require first-line therapy for pediatric ITP. In patients requiring second-line treatments, rituximab or the thrombopoietin receptor agonists have sufficient literature support, and selection may depend on patient and family characteristics and preferences. Rituximab may also be used as a second-line therapy with initial response rates of 40 to 50% but a long-term response rate of <25%. Eltrombopag, an oral thrombopoietin receptor agonist (TPO-RA), and romiplostim, a once weekly subcutaneous TPO-RA, have both been approved by the United States Food and Drug Administration (FDA) for use in children >1 year of age with chronic ITP who have failed a prior ITP therapy and require additional treatment to raise the platelet count. Randomized controlled trials in pediatrics have shown a 60 to 75% response rate with a 30% to 40% rate of sustained response. Splenectomy is generally reserved for severe persistent or chronic thrombocytopenia with bleeding and results in complete remission in ~75% of children. The risk for overwhelming sepsis after splenectomy is greater in young children, and therefore, splenectomy generally is deferred until at least 5 years of age. Vaccination against *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* type b should be given before splenectomy in children and adults, and penicillin prophylaxis is recommended until adulthood. Data from a recent randomized controlled trial of IVIG in intial treatment of newly diagnosed ITP did not alter long-term ITP outcomes and showed that observation is a safe option for most patients with newly diagnosed ITP, irrespective of platelet count. The data from this trial (called the TIKI trial), suggests that there may be benefit to treatment in patients with bleeding symptoms at diagnosis ≥ grade 3.

Management of primary ITP in adults

In contrast to children, ITP in adults evolves into a chronic disease in approximately 75% of patients. The goal of ITP management in adults is to maintain a hemostatic platelet count while minimizing the toxicity of therapy. There are no controlled studies demonstrating the superiority of any specific treatment algorithm, and significant variability exists among treatment approaches advocated by different experts. Asymptomatic patients with mild or moderate thrombocytopenia and no bleeding require no specific treatment. Platelet counts $<30 \times 10^9$/L may be associated with an increased bleeding risk. This platelet count threshold has been suggested by some experts as a cutoff for considering treatment of ITP. However, there is significant variability in bleeding among patients, and therapy should be individualized. Treatment decisions should not be dictated by the platelet count alone but should take into account other factors, including the individual patient's bleeding phenotype; the need for concomitant antithrombotic therapy or other medications that affect hemostasis; the need for an invasive procedure or surgery; lifestyle; comorbidities; and patient values and preferences, including a desire to participate in sports or other activities associated with bleeding risk. Even in asymptomatic or minimally symptomatic patients, an initial short-term treatment course is reasonable to support the diagnosis of ITP and to identify a treatment to which the patient responds in case of worsening symptoms or the need for an invasive procedure.

Although several first-line therapies are available, corticosteroids remain the initial treatment of choice because of their efficacy and low cost. At least 75% of patients initially respond to corticosteroids, although tapering usually precipitates relapse, and ultimately only 20% to 25% of patients are able to maintain a durable platelet response after steroid discontinuation. Standard corticosteroid regimens include prednisone 0.5-2 mg/kg/day and high-dose dexamethasone (40 mg daily for 4 days); available data comparing the 2 regimens demonstrate that patients receiving dexamethasone may have higher platelet counts at day 7 and, based on available data, may have increased remission rates (relative risk, 2.96; 95% confidence interval [CI] 1.03-2.96), but because of heterogeneity of the studies, a recent American Society of Hematology expert panel was not able to recommend one therapy over the other. Overall, there were no differences in response at 1 month, durable response or major bleeding, and it is also worth noting that high-dose dexamethasone may be more likely to precipitate acute corticosteroid side effects, such as psychosis in the elderly or those with a history of psychiatric illness. Approximately 25% of patients with ITP may achieve a durable remission after treatment with corticosteroids, usually within the first year after presentation. This observation has led to a recommendation by

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the International Working Group that splenectomy be deferred until at least 1 year after presentation, if possible. Importantly, the recent American Society of Hematology ITP guidelines recommend against prolonged corticosteroid use (>6 weeks) and stress the importance of monitoring for corticosteroid toxicities, including hypertension, hyperglycemia, sleep and mood disturbances, gastric irritation or ulcer formation, glaucoma, myopathy, and osteoporosis. A recent UK study (the FLIGHT trial) demonstrated that the addition of mycophenolate mofetil may improve long-term remission rates in adults when added to initial therapy of ITP.

IVIG and anti-D may be considered in patients who are bleeding (or considered high risk), in preparation for a surgical procedure, or in patients who are unresponsive to corticosteroid therapy. Anti-D (50-75 ug/kg) should only be considered in Rh-positive patients with an intact spleen who have a negative direct antiglobulin test (there is a black box warning from the FDA for risk of intravascular hemolysis in ITP with use of this agent). IVIG is generally given in a single dose (1 g/kg intravenously [IV]), on 1 or 2 days, but can be split (0.4 mg/kg) over 5 days. Both agents are associated with response rates similar to those of corticosteroids; however, the duration of response is generally only 2 to 4 weeks, and thus, frequent, intermittent dosing is required if these agents are used for chronic therapy. One uncontrolled study of 28 Rh-positive, nonsplenectomized adults reported that repeated dosing of anti-D for platelet counts $<30 \times 10^9$/L was an effective maintenance therapy and that 43% of patients treated in this manner ultimately entered a durable remission. Nevertheless, both IVIG and anti-D generally are considered to be bridging agents used to maintain platelet counts in a hemostatic range until more definitive therapy can be initiated.

Second-line therapy is indicated for patients who do not respond to first-line therapy or relapse after it is tapered. Options for second-line therapy include rituximab, TPO-RAs (eltrombopag, romiplostim, or avatrombopag), or splenectomy. In addition to eltrombopag and romiplostim, approved for both adult and pediatric ITP, avatrombopag, another oral small-molecule TPO-RA, is approved for ITP in adults. Unlike eltrombopag, avatrombopag does not require adherence to certain dietary restrictions for absorption and is not associated with hepatotoxicity. Splenectomy has been used to treat ITP for decades, although the availability of alternative treatments, concerns about adverse events, and the realization that some patients with newly diagnosed ITP ultimately may improve over time has led to decreased utilization in contemporary cohorts compared with older series. Current guidelines recommend, if splenectomy is not an option or not desired by the patient, use of TPO-RAs first after failing a first-line treatment. TPO-RAs provide a >60% response rate in patients with and without prior splenectomy, and these responses have been shown to be durable (lasting up to 6-8 years). Although there are emerging data about the potential for long-term remission in 10% to 30% of patients with TPO-RA therapy, these data are still quite immature, and most patients are expected to require ongoing treatment. If long-term remission is the goal instead of maintenance therapy, rituximab or splenectomy may be considered, with rituximab providing the potential of splenectomy-sparing therapy. Historically, splenectomy leads to a high rate of durable remission. In a systematic review, 1731 (66%) of 2623 adults with ITP achieved a complete response following splenectomy at a median follow-up of 28 months (range, 1 to 153 months), and this response rate was maintained at 10 years after splenectomy. Splenectomy does not jeopardize subsequent responses to other ITP therapies (other than anti-D) and may reduce long-term costs of ITP management. Disadvantages of splenectomy include a lack of validated predictors of response; surgical risk with a 30-day mortality and complication rate of 0.2% and 9.6%, respectively, for laparoscopic splenectomy and 1.0% and 12.9%, respectively, for open splenectomy; an increased risk of postsplenectomy infection; and an increased risk of vascular thrombosis. The incidence of infection may be reduced by presplenectomy pneumococcal, meningococcal, and *Haemophilus influenzae* type b vaccination; repeat pneumococcal vaccination 5 years after initial vaccination; and antibiotic prophylaxis for fever.

Rituximab, an anti-CD20 monoclonal antibody that rapidly depletes $CD20^+$ B lymphocytes, may be used in lieu of splenectomy or in patients who have failed splenectomy. The usual dose is 375 mg/m^2 weekly for 4 weeks, although an optimal dosing regimen has not been defined and lower doses have shown similar efficacy. In a systematic review of 313 ITP patients, half of whom were not splenectomized, 62.5% achieved a platelet count response (platelet increment of 50×10^9/L), with a median time to response of 5.5 weeks (range, 2 to 18 weeks) and a median duration of response of 10.5 months (range, 3 to 20 months). In a single-arm study of 60 nonsplenectomized ITP patients, 40% achieved a platelet count $\geq 50 \times 10^9$/L with at least a doubling from baseline at 1 year, and in 33.3%, this response was sustained for 2 years. An appealing aspect of rituximab therapy is the potential induction of long-term responses in a subset of patients, although long-term remission rates have generally been disappointing. In a long-term follow-up study, only 21% of adults treated with rituximab remained free of relapse at 5 years. In a

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recently published randomized placebo-controlled trial, there was no benefit of rituximab compared with placebo by 18 months after treatment. Adverse effects of rituximab include infusion reactions (eg, hypotension, chills, and rash), serum sickness, and cardiac arrhythmias. Reactivation of latent JC virus causing progressive multifocal leukoencephalopathy has been reported, but it appears to be extremely uncommon. Reactivation of hepatitis B after rituximab has been described, and active hepatitis B infection is a contraindication to treatment. Rituximab also interferes with the response to polysaccharide vaccines. This is of potential concern in patients who may subsequently undergo splenectomy and supports the practice of administering immunizations prior to rituximab.

Recently, the FDA approved the use of the spleen tyrosine kinase (syk) inhibitor, fostamatinib, for adult patients with chronic ITP and inadequate response to prior therapy. This was based on 2 double-anonymized randomized controlled trials in patients with longstanding ITP who had failed one or more prior treatments, demonstrating that a dose of 100-150 mg twice daily provided a 43% overall response (platelet count $>50 \times 10^9$/L). Although only 18% of patients maintained the platelet count for 4 out of 6 weeks, responses were fairly rapid (median 15 days), and the most common adverse events were hypertension and diarrhea.

Refractory ITP in adult and pediatric patients

For patients who do not respond to or are intolerant of second-line therapy, various immunosuppressant medications are available, including azathioprine, cyclosporine, mycophenolate mofetil, cyclophosphamide, vinca alkaloids, dapsone, and danazol. Evidence for use of these agents is limited to uncontrolled case series. Thrombocytopenia in patients with secondary ITP may respond to treatment of the underlying disease. For example, treatment of HIV with antiretroviral therapy induces a platelet response in most patients. Eradication of *H. pylori* has led to resolution of ITP in >50% of cases in certain countries, including Japan, although it has generally not been effective in North America. This may reflect differences in endemic *H. pylori* strains in different geographic regions.

Iron-deficiency anemia is common in ITP, particularly in menstruating women. It should be noted that eltrombopag has an off-target iron chelation effect, which may exacerbate iron deficiency in ITP patients. In addition to platelet-raising therapy, an important element of management is correction of iron deficiency and anemia because a normal red blood cell count improves hemostasis, probably through rheological factors that bring platelets into closer proximity to the endothelium in flowing blood.

Emergency treatment of ITP

Patients with new-onset, severe thrombocytopenia ($<20 \times 10^9$/L), and bleeding should be hospitalized. Examination of the peripheral blood film to exclude thrombotic microangiopathy (TMA) and a careful medication history to exclude drug-induced thrombocytopenia should be undertaken. Once a presumptive diagnosis of ITP has been reached, management of bleeding may require platelet transfusions in combination with high doses of parenteral corticosteroids (methylprednisolone 1 g intravenously daily for 2 to 3 days) supplemented with IVIG (1 g/kg for 1 to 2 days). Increases in the platelet count may become apparent within 3 to 5 days, although complete responses may require 1 to 2 weeks. Addition of antifibrinolytics may be helpful, particularly if bleeding is primarily mucosal. The bleeding patient with ITP and severe thrombocytopenia should hold antiplatelet and anticoagulant therapies, unless there is a compelling reason to continue those therapies (except in the setting of life-threatening bleeding). This aspect of bleeding management is occasionally overlooked and is an important consideration, particularly as the complexity of patients increases. TPO-RAs can be a useful adjunct, especially in patients with more refractory thrombocytopenia, although treatment typically takes 5 to 7 days to have an effect. All-*trans*-retinoic acid, vincristine, or emergency splenectomy may be required for patients with refractory thrombocytopenia and persistent bleeding. In case of life-threatening bleeding, massive platelet transfusion can control hemorrhage, but typically multiple platelet units are required.

KEY POINTS

- ITP may occur as a primary disorder or secondary to a predisposing illness.
- The diagnosis of primary ITP is made by excluding other causes of thrombocytopenia.
- ITP in children is usually self-limited; conversely, ITP in adults develops into a chronic disease in ~75% of patients.
- The pathogenesis of ITP involves accelerated platelet destruction and decreased platelet production.
- Corticosteroids, supplemented as needed with IVIG or anti-D, are first-line therapy for ITP.
- Second-line therapy (TPO-RAs, rituximab, splenectomy, and fostamatinib) is indicated in patients who do not respond to first-line therapy or relapse after it is tapered.
- Emergency treatment of severe ITP includes a combination of steroids and IVIG and, in case of life-threatening bleeding, platelet transfusion. Other agents may also be required.

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Drug-induced immune thrombocytopenia

Drugs may cause thrombocytopenia via immunologic and nonimmunologic mechanisms (such as direct bone marrow toxicity). The discussion in this chapter will focus on immunologic mechanisms. Drug-induced immune thrombocytopenia is particularly common in hospitalized, critically ill, and perioperative patients but occurs in routine outpatient settings as well. More than 300 drugs have been implicated in DITP. In clinical practice, the drugs most frequently encountered include antibiotics (trimethoprim-sulfamethoxazole, vancomycin, beta-lactams, and rifampin), anticonvulsants (such as valproate and carbamazepine), and antipsychotics. Other important agents include quinine and quinidine (present in tonic water, bitter lemon, and certain medications), nonsteroidal anti-inflammatory agents, and the platelet GPIIb-IIIa inhibitors tirofiban, eptifibatide, and abciximab. A systematic review of individual patient data found that the most commonly reported drugs with a definite or probable causal relation to thrombocytopenia were quinidine, quinine, rifampin, and trimethoprim-sulfamethoxazole. A database of implicated drugs is available online and periodically updated (Platelets on the Web; available at http://www.ouhsc.edu/platelets). Heparin-induced thrombocytopenia (HIT) is discussed separately because of its unique clinical manifestations and pathophysiology.

Mechanisms of DITP

DITP characteristically occurs approximately 1 to 2 weeks after initial drug exposure, a timeframe consistent with production of drug-dependent or drug metabolite-dependent IgG antibodies. An exception is thrombocytopenia induced by the GPIIb-IIIa antagonists eptifibatide, tirofiban, and abciximab, which may present within hours of exposure because of naturally occurring antibodies. Several mechanisms specific for individual drugs underlie the development of DITP. Quinine induced thrombocytopenia was first described more than a century ago and serves as a prototype. In this disorder, the binding of antibodies to platelet GPs is greatly enhanced in the presence of the sensitizing drug. This may result from binding of the drug to specific GPs, such as GPIIb-IIIa or GPIb-IX. Affinity maturation of B cells producing low-affinity antibodies reacting with the neoepitope induced by the complex of the drug and the platelet GP may result in the generation of antibodies that can destroy platelets in the presence of the drug. Another potential mechanism is modification of the hypervariable region of the antibody when a small-molecule drug binds to the antigen recognition site, thereby modifying the specificity of the antibody.

A much rarer mechanism of DITP involves the induction of autoantibodies by drugs such as gold and interferon-α or -β, leading to development of a syndrome that resembles ITP. An often-overlooked cause of DITP is that which follows vaccinations, including diphtheria-pertussis-tetanus and measles-mumps-rubella, which reflects the development of true autoantibodies similar to those described in ITP.

Tirofiban and eptifibatide ("fibans") are mimetics of the arginine-glycine-aspartic acid (RGD) region of fibrinogen that inhibit fibrinogen binding to activated GPIIb-IIIa and block platelet aggregation. Thrombocytopenia may occur because of preexisting antibodies that recognize conformation-dependent neoepitopes (mimetic-induced binding sites induced in GPIIb-IIIa following drug binding [rapid onset]), as well as by induction of new antibodies toward the neoepitope induced by fiban binding to the GPIIb-IIIa complex (delayed onset). Abciximab, a chimeric (mouse-human) Fab fragment to GPIIb-IIIa, causes acute profound thrombocytopenia in 0.5% to 1.0% of patients on their first exposure because of preexisting antibodies that recognize the murine portion of abciximab. About 50% of cases of "fiban-induced thrombocytopenia" are because of pseudothrombocytopenia, which, unlike typical pseudothrombocytopenia, may also manifest in citrated blood. As fibans are given in patients with high-risk coronary interventions, recognition of pseudothrombocytopenia is of major importance. Inappropriate cessation of antiplatelet therapy, and potentially even platelet transfusion or other prohemostatic measures, may subject the patient to an increased risk of thrombosis, including in-stent thrombosis.

Diagnosis of DITP

Clinical criteria have been proposed that may be used to judge the likelihood of a given drug causing DITP. These include a temporal association between drug exposure and thrombocytopenia, the exclusion of other causes of thrombocytopenia, and recurrence of thrombocytopenia upon drug rechallenge. In practice, particularly in hospitalized patients, a multitude of potential culprit drugs and concurrent illnesses (such as infections) may make the diagnosis of DITP difficult. An important diagnostic clue is the timing of initiation of the drug. Typically, the causal drug has been started 1 to 2 weeks before the onset of thrombocytopenia. In hospitalized patients, antibiotics are the most frequent cause of DITP. Specialized laboratory assays for antibodies that bind to platelets in the presence of a drug or drug metabolite have been developed. However, such

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assays are only available for a limited number of drugs and drug metabolites, are not standardized, and are only performed at a small number of reference laboratories around the world. They may provide useful confirmation of DITP, but because there is a several-day turnaround time for these "send-out" tests, clinicians are forced to make critical initial decisions about whether to suspend suspicious medications without the benefit of laboratory results.

DITP is characteristically severe, with a median nadir platelet count of approximately 20×10^9/L and a high risk of hemorrhage. A review of 247 case reports of DITP found an incidence of major and fatal bleeding of 9% and 0.8%, respectively.

Treatment for DITP involves discontinuation of the offending drug. A practical approach in hospitalized patients on multiple medications is to stop all potential culprit drugs started within the last 2 weeks that can be safely discontinued and to switch antibiotics. The platelet count generally starts to recover after 4 to 5 half-lives of the culprit drug or drug metabolite, which can last several days, especially in patients with compromised renal and hepatic function. Patients with severe thrombocytopenia and bleeding, as well as those judged to be at particularly high risk of bleeding, may be treated with IVIG, corticosteroids, or plasma exchange, although there is only limited evidence to support these interventions. Platelet transfusion is generally ineffective. Patients should be instructed to avoid the culprit drug in the future, and it should be added to their allergy list.

KEY POINTS

- Many drugs have been implicated as causes of DITP.
- Quinidine, quinine, and antibiotics, such as trimethoprim-sulfamethaxazole and vancomycin, are common culprits.
- Thrombocytopenia caused by tirofiban, eptifibatide, and abciximab may occur soon after exposure in patients not previously exposed to these drugs.
- DITP can be confirmed in some cases by demonstration of drug- (or drug metabolite) dependent, platelet-reactive antibodies in vitro; however, the turnaround time for this testing is several days and therefore clinical decisions need to be made in the absence of this information.

Heparin-induced thrombocytopenia

HIT is an immune (antibody)-mediated drug reaction caused by antibodies against multimolecular complexes of PF4 and heparin. Binding of HIT antibodies to Fc receptors on monocytes and platelets causes cellular activation; HIT antibodies also activate endothelial cells by binding endothelial cell-associated PF4. The net result is elevated levels of circulating microparticles and an intensely prothrombotic state. HIT occurs most commonly in patients receiving unfractionated heparin (UFH). The incidence of HIT in the in-hospital patient population is about 1 in 5000, but it varies widely among patient groups, with reported incidences of 0.2% to 5.0% in patients receiving UFH. The risk of HIT associated with low-molecular-weight heparin (LMWH) is 5- to 10-fold lower, and the risk is negligible with use of the heparin pentasaccharide fondaparinux. Use of LMWH instead of UFH is the most efficient measure to prevent HIT in any patient group (but LMWH must not be used when HIT has developed). Thrombosis develops in 40% to 50% of patients with HIT. Despite the occurrence of thrombocytopenia, bleeding is rare. Although the suspicion of HIT in the acute setting is clinical, confirmation depends on correlative laboratory testing. Transient thrombocytopenia following the administration of heparin (previously called type I HIT, or nonimmune HIT) is an innocuous syndrome caused by binding of heparin to platelet GPIIb-IIIa, thereby inducing a signal, which lowers the threshold for platelet activation by other agonists.

Clinical features

HIT is a clinicopathological syndrome that requires both the presence of platelet-activating antibodies, usually directed toward PF4/heparin complexes, and clinical symptoms that include a decrease in the platelet count, usually by at least 50% (median percent decrease ~75%), and/or new thromboembolic complications. Note that patients with HIT and a higher baseline platelet count may experience a drop consistent with HIT but still have a platelet count above the lower limit of the laboratory reference range. As a general rule, clinical findings manifest between day 5 and 14 after initiation of heparin. An exception is rapid-onset HIT, in which patients with recent heparin exposure (usually within the last 30 days) and preexisting HIT antibodies may manifest clinical HIT within hours of heparin reexposure. A second exception is delayed-onset HIT. In delayed-onset HIT, while the onset is actually in the usual 5- to 10-day window of classic HIT, the platelet count fall occurs after hospital discharge and is typically only recognized after a thrombotic event has occurred, leading to delays in diagnosis. In delayed-onset HIT, the antibodies have gained autoreactivity (ie, they recognize PF4 bound to endogenous glycosaminoglycans on platelets and therefore activate platelets even in the absence of heparin). A platelet

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count decrease or a new thrombosis without corresponding antibodies is not HIT. Similarly, a positive assay for platelet-activating antibodies or a positive PF4-heparin enzyme-linked immunosorbent assay (ELISA) alone, without corresponding clinical findings such as a platelet count drop or thrombosis, is not HIT.

HIT is uncommon in children and is about twice as common in females. The incidence of HIT is approximately 3-fold greater in surgical than in medical patients. While patients receiving thromboprophylaxis with UFH after major orthopedic surgery had the highest incidence of HIT (5%) in the 1990s, today, HIT is rare in this patient group. Whether this is related to the widespread use of LMWH or to other changes in surgical practice is unknown. Today, patients with cardiac assist devices and those undergoing cardiac surgery have the highest incidence of HIT (1% to 3%). Absolute thrombocytopenia (platelet count $<150 \times 10^9$/L) is not required for a diagnosis of HIT; rather, a substantial (typically >50%) decrease in the platelet count from the platelet count immediately preceding the subsequent HIT-related drop is required. This is particularly relevant to the postoperative setting, in which platelet count values typically rise to 20% to 30% above the preoperative baseline at day 8 to 10 after major surgery. Rarely, HIT can manifest as an autoimmune disorder without any exposure to heparin—so-called spontaneous HIT. PF4 binds to polyanions other than heparin, such as lipopolysaccharide on bacteria or RNA/DNA (released during major surgery), and undergoes the same changes in its conformation as when binding to heparin. These endogenous PF4-polyanion complexes are likely the trigger for spontaneous HIT. A typical characteristic of spontaneous HIT antibodies is that they activate platelets even in the absence of heparin. This feature is used to confirm spontaneous HIT.

Several clinical scoring systems have been developed to assist with determining the pretest probability of HIT. The most commonly used is the 4Ts score (thrombocytopenia, timing, thrombosis, and other; see Table 11-3). This system has been shown to have a high negative predictive value (ie, a low score is useful in ruling out HIT), but its effectiveness is limited by modest interobserver agreement and a relatively low positive predictive value. Recent studies have demonstrated that this system may be of less utility in intensive care patients, a setting in which HIT is often suspected because of the high prevalence of thrombocytopenia but is relatively uncommon with an incidence of ~0.5%. While a low 4Ts score (≤3 points) makes HIT unlikely, HIT may nevertheless be the underlying cause in 2% to 3% of patients. However, a combination of a low score and a negative PF4-heparin ELISA essentially rules out HIT.

Thrombosis is present in ~50% of newly diagnosed cases of HIT, and it develops in ~40% of patients with asymptomatic thrombocytopenia resulting from HIT within the first 10 days following heparin discontinuation if appropriate treatment is not administered. Venous thrombosis occurs twice as frequently as arterial thrombosis, although limb artery thrombosis, myocardial infarction, and microvascular thrombosis have been described. HIT-associated thrombosis occurs with increased frequency at sites of vessel injury (eg, central venous catheter–associated deep vein thrombosis [DVT]). For this reason, vascular interventional procedures (other than arterial thrombectomy) and placement of intravascular devices such as vena cava filters should generally be avoided. Adrenal infarction secondary to adrenal vein thrombosis, skin necrosis at heparin injection sites, and anaphylactoid reactions after an intravenous heparin bolus also may occur as a result of

Table 11-3 4Ts scoring system for HIT

4Ts	2 points	1 point	0 point
Thrombocytopenia	Platelet count decrease of >50% and platelet nadir ≥20×10^9/L	Platelet count decrease of 30%-50% or platelet nadir 10 to 19×10^9/L	Platelet count fall of <30% or platelet nadir $<10 \times 10^9$/L
Timing of platelet count fall	Clear onset of thrombocytopenia 5-10 days after heparin administration; or platelet decrease within 1 day, with prior heparin exposure within 30 days	Consistent with day 5-10 decrease but not clear (eg, missing platelet counts) or onset after day 10; or decrease within 1 day, with prior heparin exposure 30-100 days ago	Platelet count decrease <4 days without recent exposure
Thrombosis or other sequelae	New thrombosis (confirmed); skin necrosis (lesions at heparin injection site); acute systemic reaction after intravenous unfractionated heparin bolus	Progressive or recurrent thrombosis; nonnecrotizing skin lesions; suspected thrombosis (not proven)	None
Other causes for thrombocytopenia	None apparent	Possible	Definite

Adapted from Lo G et al, *J Thromb Haemost*. 2006;4:759-765.

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PF4/heparin antibodies. Thrombosis in unusual sites, such as cerebral sinuses, vascular grafts, fistulas, and visceral vessels, may also develop. Phlegmasia due to occlusion of the lower-extremity venous system resulting in arterial insufficiency may be observed when vitamin K antagonists are started too early in HIT (ie, prior to platelet count recovery). The resulting protein C deficiency triggers microvascular thrombosis distal to large vessel thrombosis, which may have occurred as an initial manifestation of HIT. Very severe HIT can be associated with disseminated intravascular coagulation (DIC). Patients with DIC often present with platelet counts below 20×10^9/L, whereas otherwise, a platelet count nadir of 40×10^9/L to 80×10^9/L is the more typical range for HIT.

HIT testing

Three types of tests are available for detection of HIT antibodies: PF4/heparin immunoassays (eg, PF4/heparin ELISA), rapid immunoassays, and functional assays demonstrating the ability of HIT antibodies to activate washed platelets (such as the serotonin release assay, generally considered the gold standard for diagnosis, or heparin-induced platelet activation [HIPA] test).

The sensitivity of most PF4/heparin immunoassays approaches 100%, and thus, a negative test is useful in excluding HIT. Difficulties concerning use of the PF4/heparin ELISA include long turnaround time in institutions in which it is not performed daily and a high false-positive rate, particularly in the postcardiac surgery setting. Specificity may be increased by considering the level of positivity. High ELISA reactivity correlates closely with the presence of platelet-activating HIT IgG, whereas positive functional platelet activation assays are uncommon in patients with weakly positive ELISA optical density values (0.4 to 0.9). Conversely, strongly positive ELISA optical density values (>2.0) may obviate the need for a functional assay for diagnostic confirmation in the appropriate clinical context. The use of an ELISA that detects only anti-PF4/heparin IgG, as opposed to the polyspecific ELISA that detects IgG, IgA, and IgM antibodies, also increases specificity, as may the addition of a confirmatory step performed in the presence of high heparin concentrations. Recently, rapid immunoassays—automated tests for anti-PF4/heparin antibodies—have been introduced. They are highly standardized and allow a rapid turnaround time, but some may produce false negative results in a significant minority of patients.

Functional assays have improved specificity compared with immunoassays. These assays are technically difficult, however, requiring washed donor platelets; and for the serotonin release assay, radioisotope. Because of these considerations, the performance of functional assays is limited primarily to specialized reference laboratories, and their results generally are not available at the time the diagnosis of HIT is considered. They are, however, important for confirming the diagnosis in many patients and for long-term management because patients without HIT may be harmed by being incorrectly labeled as having a history of HIT, with consequent avoidance of heparin and unnecessary use of alternative anticoagulants.

Treatment of HIT

Although previously underdiagnosed, increased appreciation of HIT and the frequent use of highly sensitive tests has led to overdiagnosis in the current era, with the attendant costs and increased bleeding risks associated with inappropriate anticoagulation therapy. Current guidelines from the American Society of Hematology suggest that routine monitoring of the platelet count in patients on heparin therapy should be performed at least every other day for patients with a high risk of HIT (>1%) and every 2 to 3 days in those with an intermediate risk (0.1%-1%) and that routine monitoring is unnecessary for those in whom the risk of HIT is low (<0.1%). The approximate risk of HIT according to patient population and type of heparin exposure is given in Table 11-4. However, platelet count monitoring rarely helps to prevent initial thrombosis in HIT because the time between the fall in platelet count and onset of thrombosis can be very short or both may occur concomitantly.

Table 11-4 Incidence of HIT according to patient population and type of heparin exposure

Patient population (minimum 4 days' exposure)	Incidence of HIT (%)
Postoperative patients	
Heparin, prophylactic dose	1-5
Heparin, therapeutic dose	1-5
Heparin, flushes	0.1-1.0
LMWH, prophylactic or therapeutic dose	0.1-1.0
Cardiac surgery patients	1-3
Medical	
Patients with cancer	1.0
Heparin, prophylactic or therapeutic dose	0.1-1.0
LMWH, prophylactic or therapeutic dose	0.6
Intensive care patients	0.4
Heparin, flushes	<0.1
Obstetric patients	<0.1

Adapted from Linkins LA et al, *Chest*. 2012;141(suppl 2):e495S-e530S (© 2012), with permission from the American College of Chest Physicians.

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The cornerstone of HIT therapy is immediate discontinuation of heparin when the disease is suspected, usually before laboratory diagnosis. Given the high rate of clinically silent DVT in HIT, 4-limb doppler ultrasound is recommended in all patients with HIT as this will influence the duration of anticoagulation. Anticoagulation using a nonheparin anticoagulant at a therapeutic dose should be initiated, even in patients with no thrombosis, because of the massive thrombin generation in HIT and continued high risk of thrombosis after heparin discontinuation. Because the hypercoagulable state is substantial, occurrence of thrombosis even after initiation of alternative anticoagulation is not uncommon. Alternative anticoagulation in patients without thrombosis should be continued until the platelet count has recovered and is typically continued for 4 to 6 weeks thereafter in patients without thrombosis, although no controlled data demonstrating the benefit of this approach are available. Patients with HIT and thrombosis should receive at least 3 months of therapeutic dose anticoagulation. LMWH must not be used because of cross-reactivity with most heparin-dependent antibodies. Warfarin must not be given in acute HIT. It may be started once the platelet count has reached a stable plateau within the normal range, indicating that the acute prothrombotic process is under control, but only with appropriate overlap with an alternative parenteral anticoagulant. Because of its inhibition of protein C γ-carboxylation, warfarin increases risk of microthrombosis in the setting of HIT-associated hypercoagulability, including risk of venous limb gangrene in a patient who has HIT-associated DVT. In patients diagnosed with HIT while taking warfarin, warfarin should be discontinued, an alternative nonheparin anticoagulant should be initiated, and vitamin K should be administered with the goal of repleting protein C and preventing microvascular thrombosis.

Currently available nonheparin anticoagulants in the United States include the parenteral direct thrombin inhibitors, argatroban and bivalirudin, as well as fondaparinux and the direct oral anticoagulants. Argatroban is hepatically cleared and approved for treatment of HIT with or without thrombosis, as well as percutaneous coronary intervention in patients with HIT or at risk for HIT. Argatroban is monitored using the activated partial thromboplastin time (aPTT) but also raises the prothrombin time/international normalized ratio. Thus, transitioning patients from argatroban to warfarin should be performed by following the guidelines suggested by the manufacturer. Bivalirudin is approved for percutaneous coronary interventions in patients with HIT or a history of HIT and has the advantage of a short half-life of only 25 minutes. A limitation of both argatroban and bivalirudin is that they are subject to aPTT confounding, a phenomenon in which patients with clotting factor deficiency (due to liver impairment, warfarin treatment, or consumptive coagulopathy) have resultant prolongation of the aPTT, leading to underdosing of anticoagulation. Use of the dilute thrombin time assay rather than the aPTT provides more reliable results. Other anticoagulants, such as danaparoid and lepirudin, are no longer available in the United States.

A number of reports have described the favorable use of the synthetic heparin pentasaccharide fondaparinux in patients with HIT, although this agent has not been studied in a controlled manner. The main consideration with use of fondaparinux in acutely hospitalized patients with HIT is its long half-life (20 hours). The direct oral FXa inhibitors (rivaroxaban, apixaban, and edoxaban) or the direct oral thrombin inhibitor dabigatran may be an option in HIT, but apart from case series, no controlled data are available. Advantages to these agents include fixed dosing and avoidance of aPTT confounding. Because the plasma levels of direct oral FXa inhibitors and dabigatran change considerably between peak and trough; however, there is a risk that the highly prothrombotic state of acute HIT could lead to breakthrough thrombosis at drug trough levels. The oral direct thrombin and FXa inhibitors may be used once the acute phase of HIT is resolved (as signified by platelet count recovery).

In patients with autoimmune HIT, alternative anticoagulants must be maintained in therapeutic dose until the platelet count has reached normal levels, which may last several months. Recently, several patients with autoimmune HIT have been shown to respond with a rapid and persistent increase of the platelet count upon treatment with high-dose IVIG (1 g/kg/day for 2 consecutive days). In part, high-dose IVIG blocks activation of the platelet Fc receptor by HIT antibodies, but it may also have an immune-modulatory effect. IVIG does not have anticoagulant activity and so it must be given with a nonheparin anticoagulant.

HIT antibodies are transient and typically vanish within 3 months after discontinuation of heparin. Once antibodies disappear (ie, HIT laboratory testing becomes negative), it is safe to re-expose patients to heparin during a cardiovascular procedure or surgery. Heparin must be limited to the intraoperative setting and scrupulously avoided before and after surgery. If cardiovascular surgery is required in a patient with HIT and the procedure cannot be delayed until HIT antibodies disappear, options for intraoperative anticoagulation include use of a nonheparin parenteral anticoagulant (eg, bivalirudin), plasma exchange (using plasma as the replacement fluid)

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to reduce HIT antibody titers and allow heparin use, or use of intraoperative heparin in combination with a prostacyclin analogue.

Vaccine-induced immune thrombotic thrombocytopenia

Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a newly described HIT-like prothrombotic syndrome that is a rare and potentially fatal complication of adenoviral vector vaccines directed against SARS-CoV-2, the virus causing coronavirus disease 2019 (COVID-19). VITT is also known as thrombosis and thrombocytopenia syndrome and was previously called VIPIT (vaccine-induced prothrombotic immune thrombocytopenia). VITT results from non-heparin-dependent IgG antibodies that can bind PF4 in association with platelets and cause platelet activation. Venous and/or arterial thrombosis and thrombocytopenia occurring approximately 5 to 20 days from the date of vaccination are the cardinal features of the syndrome, although delays in seeking medical attention may mean a longer duration from vaccination to diagnosis. Therefore, clinicians must remain vigilant in recognizing the clinical features of the syndrome in any patient with recent receipt of an adenoviral vector COVID-19 vaccine. While patients with VITT can present with DVT in the extremities or with pulmonary emboli, they frequently develop venous thrombosis in unusual sites, such as cerebral or splanchnic veins. In reported cases, the platelet count at diagnosis is typically in the range of 20,000 to 80,000/μL but can be slightly lower or higher (even above 100,000/μL). Thrombocytopenia in VITT has been associated with life-threatening bleeding, and patients may also present with disseminated intravascular coagulation.

Patients with suspected VITT should have laboratory testing (platelet count, PF4 antibody ELISA, D-dimer, prothrombin time, partial thromboplastin time, and fibrinogen) performed immediately, along with imaging to diagnose thrombosis or bleeding based on presenting symptoms. Of note, rapid HIT diagnostic assays are not a substitute for a PF4 antibody ELISA test in VITT diagnosis as they are unreliable in this setting. Management includes anticoagulation with a nonheparin anticoagulant (in all patients without catastrophic bleeding) and IVIG. There may also be a role for therapeutic plasma exchange in patients unresponsive to anticoagulation and IVIG.

To date, VITT has only been conclusively linked to the ChAdOx1 CoV-19 vaccine (AstraZeneca) and the Ad26.COV2.S vaccine (Johnson & Johnson/Janssen), both adenoviral vector vaccines, and has not been observed in patients receiving other COVID-19 vaccines (such as the mRNA-based vaccines from Pfizer-BioNTech or Moderna). The precise incidence of VITT is not clear at this time but is believed to be low (ranging from 1:25,000 to 1:200,000), with younger individuals and women possibly at greater risk. Most international public health authorities have concluded that the benefits of vaccination with either of these vaccines far outweighs the risk of VITT, especially while the COVID-19 pandemic remains ongoing worldwide.

KEY POINTS

- HIT occurs in 0.2% to 5% of adults exposed to UFH, approximately 40% to 50% of whom develop thrombosis.
- HIT antibodies are directed against large multimolecular complexes of PF4 and heparin (or other polyanions).
- Systematic scoring systems facilitate estimation of the pretest probability of HIT. A low 4T score (≤3 points) makes HIT very unlikely and, together with a negative PF4-heparin ELISA, rules out HIT.
- Functional assays including the serotonin release assay and the HIPA test are useful for confirming the diagnosis of HIT.
- When HIT is suspected, heparin must be discontinued and a nonheparin anticoagulant initiated in therapeutic dose (unless there is a substantial risk for bleeding).
- Warfarin must not be started in acute HIT but may be initiated for long-term anticoagulation once the platelet count has normalized at a stable plateau for 2 consecutive days.
- In patients diagnosed with HIT while receiving warfarin, the warfarin should be discontinued, an alternative nonheparin anticoagulant initiated, and vitamin K given to reverse warfarin effect without delay.
- Vaccine-induced immune thrombotic thrombocytopenia is a rare but potentially fatal HIT-like prothrombotic syndrome associated with adenoviral vector vaccines for SARS-CoV2.

Thrombotic microangiopathies

Clinical features

The thrombotic microangiopathies discussed in this chapter include thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome (HUS), which includes Shiga toxin, Shiga-like toxin–associated HUS (STEC-HUS), and complement-mediated HUS. Each of these disorders is characterized by MAHA and thrombocytopenia, with a variable component of neurologic or renal dysfunction and fever. This pentad of symptoms was once common at the time of presentation, but increased awareness of these disorders has led to earlier diagnosis and, as such, most patients

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are diagnosed once 2 or 3 symptoms are present. Currently, the presence of microangiopathic anemia (schistocytes and anemia) and thrombocytopenia is sufficient for the diagnosis of thrombotic microangiopathy (Table 11-5).

TTP occurs in both a congenital (cTTP) form (classically called Upshaw-Schulman syndrome) due to biallelic mutations in the VWF-cleaving protease, ADAMTS13 (a disintegrin and metalloprotease with thrombospondin-1-like repeats, member 13) in <5% of cases, as well as a more common (95% of cases) immune-mediated (iTTP) form, in which ADAMTS13 deficiency is caused by autoantibodies. In some groups, however, such as young children and pregnant women, cTTP may represent 25% to 50% of all patients.

Complement-mediated HUS comprises many forms of HUS, including many secondary forms of HUS (transplant-associated, lupus-associated, catastrophic antiphospholipid antibody syndrome–related, vasculitis-related, paroxysmal nocturnal hemoglobinuria-associated, and medication-induced) and atypical HUS (aHUS) caused in up to 70% of patients by pathogenic variants in complement (regulatory) genes: complement factor H (CFH), membrane cofactor protein (MCP; CD46), factor I, C3, and thrombomodulin. Approximately 10% of aHUS patients have autoantibodies against CFH (predominantly adolescent patients). Patients with TTP or HUS may present with overlapping symptoms of fatigue and malaise, variable neurologic symptoms ranging from mild personality changes to obtundation or seizures, and variable renal insufficiency. Laboratory findings of MAHA and thrombocytopenia; changes in creatinine, international normalized ratio (commonly known as INR), or mean corpuscular volume; or evidence of hemolysis all contribute to a clinical score called the PLASMIC score, which is helpful in determining the likelihood of TTP, and additional diagnostic testing may also help differentiate the types of TMAs.

Table 11-5 Classification scheme for thrombotic microangiopathies

Disorders in which etiology is established
ADAMTS13 abnormalities
ADAMTS13 deficiency secondary to mutations
Antibodies against ADAMTS13
Disorders of complement regulation
Genetic disorders of complement regulation
Acquired disorders of complement regulation (eg, factor H antibody)
Infection induced
Shiga toxin– and verotoxin (Shiga-like toxin)–producing bacteria
Neuraminidase related: *Streptococcus pneumoniae,* influenza
Defective cobalamin metabolism
Quinine induced
Disorders in which etiology is not well understood
HIV
Malignancy
Drugs
Pregnancy
Systemic lupus erythematosus and antiphospholipid syndrome

Adapted from Taylor CM et al, *Br J Haematol*. 2009;148:37-47; based on Besbas N et al, *Kidney Int*. 2006;70:423-431 (© 2006), with permission from Elsevier.

Pathogenesis

TMAs cause microvascular thrombi in critical organs, leading to ischemia and organ damage. These thrombi induce shearing of red blood cells, leading to the characteristic anemia with schistocytes. Endothelial cell activation or damage also promotes TMA, leading to the elaboration of unusually large VWF multimers that enhance platelet aggregation and microvascular occlusion.

TTP results from an inherited or acquired deficiency of ADAMTS13, leading to elevated levels of unusually large VWF multimers that induce platelet aggregation in the microvasculature. ADAMTS13 regulates VWF activity by cleaving high-molecular-weight multimers; failure to do so may result in the microvascular thrombosis and ischemia characteristic of TTP (Figure 11-3). The observation that some patients with ADAMTS13 deficiency do not have clinical manifestations of TTP suggests that factors other than ADAMTS13 deficiency, such as endothelial damage or activation, are also needed to trigger TTP. Other TTP-like syndromes can be caused by drugs—including quinine, ticlopidine, clopidogrel, cyclosporine, tacrolimus, mitomycin C, and gemcitabine—or may occur in the setting of bone marrow transplantation, systemic lupus erythematosus, disseminated malignancy, and HIV infection. The pathogenesis of these syndromes is diverse; whereas some are associated with antibodies to ADAMTS13, others are not and may result from direct endothelial cell toxicity. Many of these other disorders are associated with evidence of terminal complement activation such as elevated soluble C5b-C9 typical of aHUS as well as modest decreases in ADAMTS13 activity levels without severe deficiency.

STEC-HUS results from infection by enteropathogenic *Escherichia coli*. The capacity of organisms to cause HUS reflects their production of two 70-kDa bacterial exotoxins called verotoxins. Verotoxin-1 is homologous to a *Shigella* toxin and therefore generally is referred to as Shiga-like toxin 1 (SLT-1 or Stx1). Most strains of pathogenic *E. coli* produce a second toxin, Stx2, which is associated with a higher risk of developing HUS. The intact 70-kDa Stx holotoxin consists of a 32-kDa A subunit

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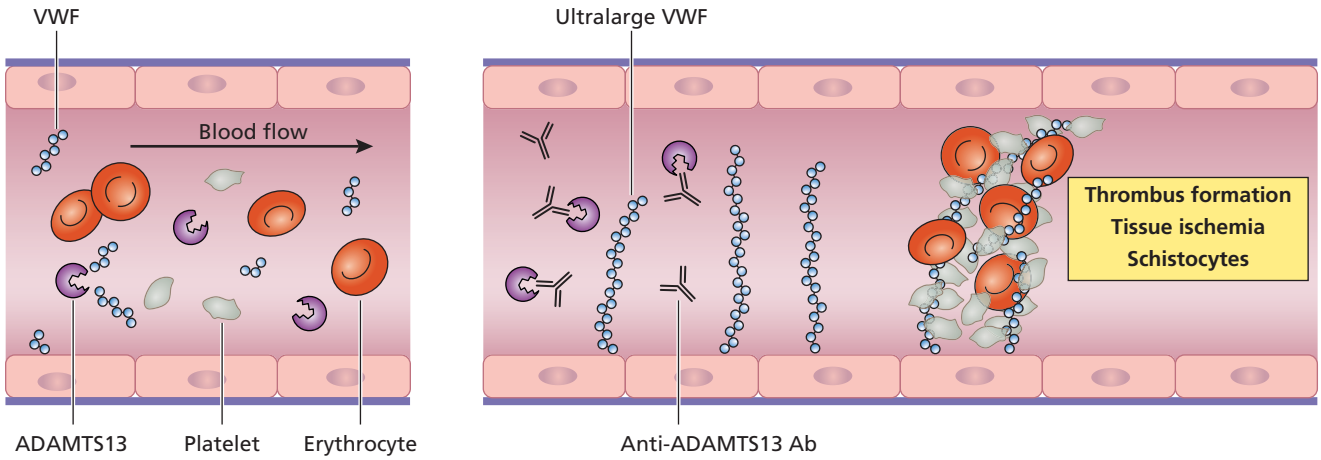

Figure 11-3 Pathogenesis of TTP caused by ADAMTS13 deficiency. Multimeric VWF adheres to endothelial cells or to connective tissue exposed in the vessel wall. Platelets adhere to VWF through platelet membrane GPIb-IX. In flowing blood, VWF in the platelet-rich thrombus is stretched and cleaved by ADAMTS13, limiting thrombus growth. If ADAMTS13 is absent, VWF-dependent platelet accumulation continues, eventually causing microvascular thrombosis and TTP. Adapted from Azoulay et al, *Intensive Care Med*. 2019;45(11):1518-1539.

and five 7.7-kDa B receptor-binding subunits that bind globotriaosylceramide (Gb3; CD77) receptors expressed on capillary endothelium. Following binding to Gb3, the toxin is internalized. The A subunit is proteolyzed to a 27-kDa A1 subunit that binds the 60s ribosomal subunit, inhibiting protein synthesis and inducing endothelial cell apoptosis. Recent studies have demonstrated that signal transduction initiated through cross-linked Stx B subunit/Gb3 complexes induce the release of VWF from endothelial cells. Finally, Stx acts in concert with lipopolysaccharide to trigger a procoagulant state that involves platelet activation, tissue factor induction, and the release of unusually large VWF multimers.

The pathogenesis of aHUS reflects increased activation of the alternative complement pathway (AP) because of mutations or autoantibodies resulting in loss or functional impairment of complement regulatory proteins or, less frequently, activating mutations in complement proteins themselves. Most hereditary forms of aHUS are transmitted in an autosomal dominant manner, although penetrance is only 50%. Under normal conditions, the AP is constitutively activated because of ongoing C3 hydrolysis (Figure 11-4), and thus, tight regulation of the AP by complement inhibitory proteins is required to prevent complement-mediated injury. AP activation leads to the generation of the C5b-C9 lytic complex on cell surfaces, and in the case of aHUS, endothelial cell damage is the primary consequence, resulting in characteristic microvascular thrombotic lesions. Complement activation is regulated primarily by the plasma protein, factor H, and the membrane-associated membrane cofactor protein (MCP; CD46), each of which binds membrane-bound C3b and promotes its inactivation by factor I. Several mutations in complement regulatory proteins underlie the development of aHUS. Most common are mutations in factor H, which impair the interactions of factor H with membrane-bound C3b and account for 30% of cases; an additional 5% to 10% of cases of aHUS result from acquired antibodies to factor H. Mutations in CD46, usually impairing membrane expression, are observed in 15% of patients with aHUS. Factor I mutations occur in 12% of aHUS patients. Activating mutations in factor B or C3 occur in 5% to 10% of patients with aHUS. Mutations in thrombomodulin, another complement regulatory protein, have been described.

Diagnosis

The diagnosis of TMA requires clinical awareness and prompt recognition of symptoms (Figure 11-5). TTP is more common in females, with a peak incidence in the fourth decade; other risk factors include obesity and African ancestry. The diagnosis of TTP should be suspected in patients with MAHA and thrombocytopenia without another apparent etiology, such as malignant hypertension, vasculitis, scleroderma renal crisis, tumor emboli, or DIC. Fever and neurologic symptoms may be present but are less common than they once were because of earlier diagnosis; evidence of renal involvement even in the absence of renal insufficiency sometimes can be obtained through examination of the urinary sediment. Schistocytes are common but not necessary for the diagnosis and accompanied by elevation of the lactate

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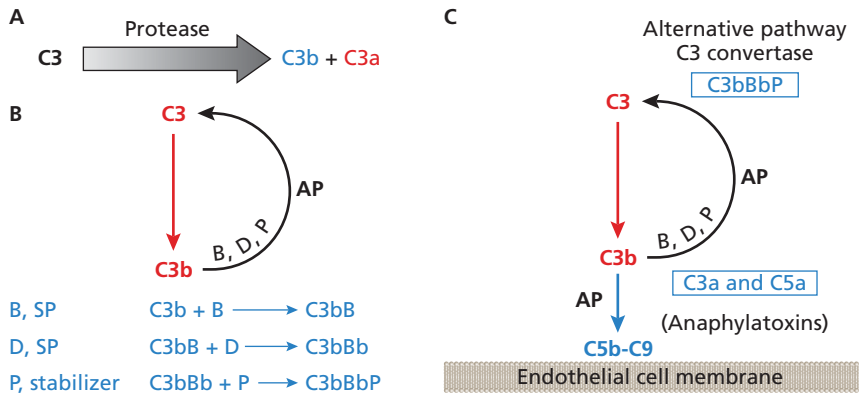

Figure 11-4 The alternative pathway (AP) of complement activation. (A) The AP of the complement system originally consisted of a serine protease that cleaved C3 to the opsonin C3b and the proinflammatory anaphylatoxin C3a. (B) An amplification loop was next evolved to more efficiently deposit C3b on a target and liberate C3a into the surrounding milieu. B indicates factor B, D indicates factor D, a serine protease; P, properdin, a stabilizer of the enzyme. (C) Development of a C5 convertase. The same enzyme that cleaves C3 (AP C3 convertase) can cleave C5 to C5a and C5b with the addition of a second C3b to the enzyme complex (AP C5 convertase). Adapted from Liszewski MK, Atkinson JP, *Hematology (Am Soc Hematol Educ Program)*. 2011;2011:9-14.

dehydrogenase, which may be striking; levels of unconjugated bilirubin also may be increased. Nucleated red blood cells are frequently present. The PT, aPTT, and fibrinogen levels are typically normal, and the D-dimer is normal or only mildly increased. The direct antiglobulin test is negative. Consideration of secondary causes of TTP should include a detailed drug history, HIV testing, and a focused search for autoimmune disease and malignancy (driven by patient signs and symptoms and certain screening labs). TTP may present during pregnancy, particularly in the second and third trimesters, and acquired TTP is very rare in children. ADAMTS13 activity assays are useful in confirming the diagnosis of TTP: severe deficiency (<10%) in a patient with a high pretest probability of TTP confirms the diagnosis versus an ADAMTS13 activity of >20% should make the treating clinical team reconsider alternative diagnoses; this does not necessarily mean that plasma exchange is not appropriate, just that the diagnosis of TTP may not be correct and an alternative explanation for the TMA should be explored. ADAMTS13 testing may also provide prognostic information, with lower levels of ADAMTS13 and higher levels of anti-ADAMTS13 antibodies associated with higher relapse rates.

Patients with aHUS may present acutely, mimicking TTP or, in some cases, more insidiously with renal insufficiency as the primary symptom. Thrombocytopenia may be less severe in aHUS than TTP. A family history of similar disease may be apparent, although the low penetrance of complement inhibitor mutations may make such a history difficult to dissect. Exacerbations of disease may follow infections and may be accompanied by fatigue and malaise. aHUS may present in association with pregnancy, most commonly at 3 to 4 weeks postpartum. Complement levels (C3 and C4) in patients with aHUS may be decreased, but normal levels do not exclude aHUS. However, all patients should have evidence of terminal complement activation and elevated soluble C5b-C9 levels. Sequencing of complement inhibitor proteins is useful for confirming a clinical impression of aHUS and may help guide long-term management, but 30% to 40% of patients who respond to complement inhibition do not have an identifiable mutation, and genetic variants of unknown significance are common.

HUS caused by Shiga toxin producing *E. coli* (STEC-HUS) or more rarely other pathogens through complement activation (pneumococcus: *Streptococcus pneumoniae*-associated HUS; viral infections: influenza virus, adenovirus, coxsackievirus, and HIV) is more common in the pediatric population than in adults and is the most common cause of acute renal failure in children. The disease begins with abdominal pain and watery diarrhea 2 to 12 days after toxin exposure. Bloody diarrhea generally ensues on the second day, although up to one third of patients do not report blood in the stool. Fever is typically absent or mild. The presentation may be difficult to differentiate from inflammatory bowel disease, appendicitis, ischemic colitis, or intussusception. Definitive diagnosis is made by culture of *E. coli* on sorbitol-MacConkey agar.

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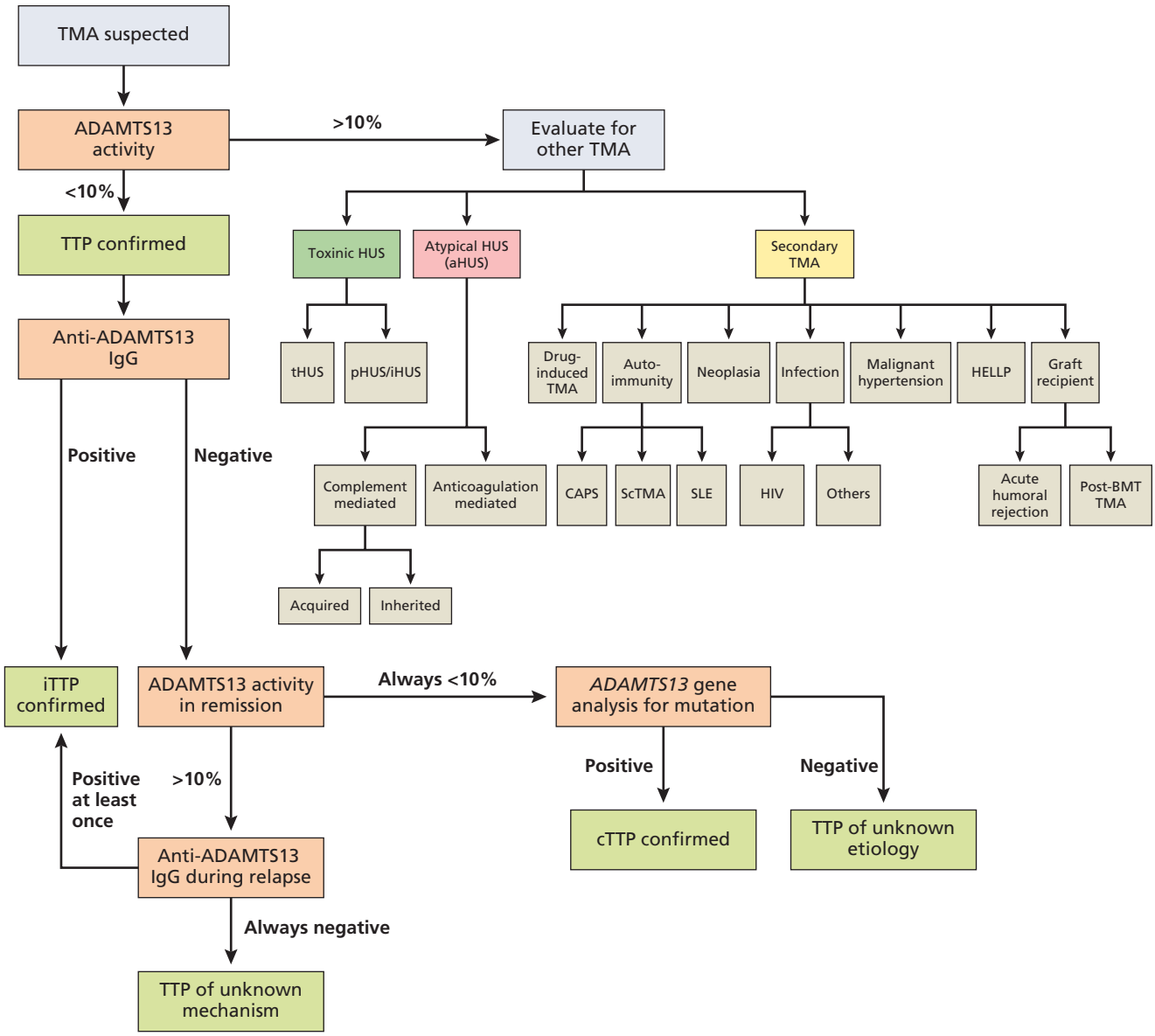

Figure 11-5 Schematic representation of the types of thromboic microangiopathy. Evaluation of thrombotic microangiopathy, including TTP, atypical HUS, and other different reasons for thrombotic microangiopathy. Adapted from Sukumar S et al, *J Clin Med*. 2021;10(3):536.

The presence of Shiga toxin or its structural genes may be detected by enzyme immunoassay or polymerase chain reaction of the stool. Serologic studies demonstrating an increase in convalescent antibody titer to Shiga toxin or *E. coli* lipopolysaccharide may be useful in confirming the diagnosis.

Management

Although plasma exchange is the standard of care for treatment of TTP, this therapy is associated with high long-term rates of renal failure in aHUS, which requires complement blockade, most commonly with monoclonal antibodies against C5 (there are now several available products that are FDA approved). Therefore, differentiating between the 2 entities of aHUS and TTP is important. Untreated, TTP is associated with a mortality of approximately 85%, although 90% of patients with TTP treated with plasma exchange survive. Initial therapy for both (because of clinical overlap) is often plasma exchange; the exchange of 1 plasma volume daily is standard initial treatment. Plasma exchange is continued daily until the platelet count reaches normal levels and symptoms have

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resolved. Neurologic symptoms improve most rapidly. No evidence suggests a benefit of either abrupt discontinuation or tapering of plasma exchange. However, samples should be sent to evaluate ADAMTS13 levels and complement activation prior to initiation of plasma exchange, and once these levels are available, if the ADAMTS13 level is >20% and/or there is evidence of complement activation, therapy with complement blockade should be considered (see the following). Corticosteroids are recommended according to the most recent International Society of Thrombosis and Haemostasis guidelines for management of presumed TTP because of the presence of ADAMTS13 antibodies, and in patients with a high level of suspicion for iTTP, or where the diagnosis is confirmed, rituximab is recommended in the initial episode. Platelet transfusion has been associated with a rapid decline in clinical status in occasional patients and is relatively contraindicated. Caplacizumab is an anti-VWF nanobody developed for treatment of TTP, which was recently FDA approved. In a phase III trial, caplacizumab added to plasma exchange shortened the time to platelet response and reduced the composite outcome of TTP-related death, TTP recurrence, and major thromboembolic events compared with plasma exchange alone. The major side effect is bleeding due to depletion of VWF. The most recent international recommendations make a conditional recommendation for the addition of caplacizumab in the initial therapy of confirmed iTTP, but the therapy is very expensive and not available everywhere as well as requires some expertise to determine optimal duration. Additionally, a recent cost-effectiveness analysis found that addition of caplacizumab to the standard of care may not be cost-effective due to high cost of the medication and its failure to improve relapse rates. Therefore, caplacizumab treatment should be considered with the guidance of clinicians experienced with its risks and benefits.

Response rates to plasma exchange in patients with aHUS are significantly worse than for TTP. Current recommendations are to initiate C5 complement blockade as soon as possible in the setting of aHUS when the diagnosis is suspected because delays in the initiation of complement blockade are associated with worse long-term renal function and a greater likelihood of dialysis dependence. Therefore, C5 complement blockade should be initiated promptly in patients with TMA who do not have severe deficiency of ADAMTS13 or another apparent cause of TMA and who do not respond to plasma exchange and not be delayed until complement mutation results are available (turnaround time for this testing is generally several weeks, and 30%-40% of patients without identifiable mutations benefit from complement blockade). Although the current standard of care is to continue complement blockade indefinitely, there is mounting evidence to suggest that it may be safe to discontinue treatment (with close surveillance) in selected patients.

Treatment of STEC-HUS is generally supportive, although there are case reports of use of complement blockade in refractory or severe cases. It was long assumed that the use of antibiotics may lead to increased toxin release and a worse outcome. However, during an epidemic outbreak of STEC-HUS, antibiotic treatment was associated with reduced morbidity. Some patients may require transfusion support and/or dialysis during the acute phase of their illness. A benefit for plasma exchange in STEC-HUS has not been demonstrated. Complement modulation may also be indicated in TMAs associated with hematopoietic stem cell transplant, chemotherapy, and in other clinical scenarios and clinical trials to evaluate that the efficacy and specific clinical indications are ongoing.

KEY POINTS

- TTP, aHUS, and STEC-HUS share many common features and may be difficult to distinguish from one another.
- The pathogenesis of TTP involves deficiency of ADAMTS13, usually because of acquired autoantibodies against ADAMTS13. This leads to accumulation of ultralarge VWF multimers that induce platelet aggregation in the microcirculation.
- aHUS involves excessive complement activation, requiring complement blockade to arrest the process.
- The pathogenesis of typical HUS reflects the effects of Shiga toxin on vascular endothelium and other cell types.
- The treatment of choice for iTTP is plasma exchange, supplemented with corticosteroids and rituximab. Addition of caplacizumab may be considered if appropriate expertise is available.
- Complement blockade (using anti-C5 antibody therapy) should be initiated as quickly as possible in suspected aHUS.
- Plasma exchange is not effective in STEC-HUS, which is usually self-limited. Treatment is supportive.

Thrombocytopenia of chronic liver disease

Patients with advanced chronic liver disease frequently develop mild-to-moderate thrombocytopenia, with platelet counts typically in the 40 to 120 $\times$ 10^9/L range. The thrombocytopenia of chronic liver disease is typically multifactorial in etiology, resulting from a reduction

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in TPO production by the diseased liver, a low-grade chronic consumptive process due to increased thrombin generation with primary hyperfibrinolysis, and a component of splenic sequestration in patients with liver cirrhosis and portal hypertension. Splenic enlargement results in sequestration of platelets in the splenic vascular network. Patients with thrombocytopenia of chronic liver disease, in particular those with more severe thrombocytopenia than would be expected for the degree of liver impairment, may have additional complicating mechanisms of thrombocytopenia associated with liver disease. Most commonly, the complicating mechanism is immunologic, such as a secondary ITP precipitated by hepatitis C infection or occurring in association with autoimmune hepatitis.

Most patients with thrombocytopenia of chronic liver disease do not develop thrombocytopenia significant enough to require intervention. In patients with more significant thrombocytopenia (platelet counts $<50 \times 10^9$/L) who require a surgical procedure, thrombopoietin receptor agonists may be given over a short preoperative course to raise the platelet count in the perioperative setting. Two small-molecule oral thrombopoietin receptor agents, avatrombopag and lusutrombopag, are currently FDA approved for this indication on the basis of randomized phase III trials (ADAPT-1 and ADAPT-2 for avatrombopag and L-PLUS 1 and L-PLUS 2 for lusutrombopag). In these randomized trials, both agents had similar efficacy in raising the platelet count in patients with chronic liver disease, thereby obviating the need for peri-procedural platelet transfusion, with minimal side effects. Importantly, elevated rates of venous thromboembolic complications, including splanchnic vein thromboses, were not observed in patients receiving avatrombopag or lusutrombopag in these trials. By contrast, a randomized trial evaluating eltrombopag for similar indications (ELEVATE) was stopped prematurely because of excessive portal vein thrombosis in the eltrombopag arm.

KEY POINTS

- Patients with chronic liver disease often have chronic, mild-to-moderate thrombocytopenia because of reduced production of TPO and splenic sequestration.
- Patients with thrombocytopenia of chronic liver disease who require a surgical procedure can be treated with a short course of the thrombopoietin receptor agonists avatrombopag or lusutrombopag prior to surgery to raise the platelet count in preparation for their procedure.

Thrombocytopenia associated with infection and critical illness

Mild and transient thrombocytopenia occurs with many systemic infections. Thrombocytopenia may be caused by a combination of mechanisms, including decreased platelet production, increased destruction, and increased splenic sequestration. In viral infections, infection of megakaryocytes may lead to suppression of platelet production; in rickettsial infections, platelets may be consumed in vasculitic lesions; and in bacteremia, platelet consumption may result from DIC or enhanced clearance of immune complex-coated platelets. HIV, hepatitis C virus, and *H. pylori* are causes of secondary ITP. Hemophagocytic syndromes, which are commonly triggered by infection, may complicate infections and result in multiple cytopenias, including severe thrombocytopenia, as well as a consumptive coagulopathy. Infection-associated thrombocytopenia may be difficult to specifically diagnose given that antibiotics commonly result in DITP, and patients with critical illness develop thrombocytopenia because of other etiologies, such as extracorporeal circuits and DIC. DIC is discussed in greater detail in Chapter 2.

Approximately 40% of patients in medical or surgical intensive care units (ICUs) develop a platelet count $<150 \times 10^9$/L; 20% to 25% develop a platelet count $<100 \times 10^9$/L; and 12% to 15% develop severe thrombocytopenia with a platelet count $<50 \times 10^9$/L. The development of thrombocytopenia in patients in the ICU is a strong independent predictor of mortality. The spectrum of disorders that cause thrombocytopenia in this setting is extensive and includes DITP, infection, DIC, surgery, hemodilution, extracorporeal circuitry/intravascular devices (eg, cardiopulmonary bypass, intra-aortic balloon pumps, and extracorporeal membrane oxygenation), and HIT, among others. Management is highly dependent on the etiology of thrombocytopenia. For example, whereas platelet transfusion and suspension of anticoagulant prophylaxis may be indicated in a patient with DITP, HIT requires prompt initiation of an alternative nonheparin anticoagulant and constitutes a relative contraindication to platelet transfusion. Treatment must therefore be individualized to the underlying cause of thrombocytopenia and any concomitant hemorrhagic or thrombotic risk factors the patient may harbor. High-quality evidence linking a platelet count threshold with bleeding risk in ICU patients is lacking. Prophylactic platelet transfusion is gen- erally given when the platelet count decreases to $<10 \times 10^9$/L – 20×10^9/L. Patients with bleeding or a planned invasive procedure may require a higher platelet count. Other aspects pertaining to thrombocytopenia in critically ill patients are discussed in Chapter 2.

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KEY POINTS

- Infection is a common cause of thrombocytopenia, particularly in ICU patients, and can be induced by a variety of organisms; this etiology of thrombocytopenia is covered in greater detail in Chapter 2.
- Thrombocytopenia in the ICU may arise from a number of etiologies. Treatment should be individualized based on the etiology of thrombocytopenia and individual patient factors.

Hereditary thrombocytopenia

Hereditary thrombocytopenic syndromes are uncommon but not as rare as once assumed. A recent study demonstrated that up to 30% of adults of ITP are misdiagnosed as ITP and have a congenital thrombocytopenia. The list of inherited platelet disorders is constantly expanding with >60 genes now known to be associated with the inherited disorders of platelet number and function. It is critical that treating physicians maintain a high index of suspicion for these disorders, as patients are often misdiagnosed as having ITP, resulting in unnecessary, ineffective, and potentially harmful treatments such as immunosuppression and splenectomy. In affected families, it is common to elicit a splenectomy history in at least one family member to treat "ITP." The diagnosis should be considered in any patient with a family history of thrombocytopenia, in patients with long-lasting "ITP" who do not respond to standard therapy, or when there is bleeding out of proportion to the degree of thrombocytopenia, suggesting a concomitant platelet function disorder (eg, an intracranial hemorrhage in an "ITP" patient with a platelet count of 60×10^9/L).

Whenever possible, physicians should attempt to document a historical normal platelet count in a patient with thrombocytopenia to exclude a hereditary thrombocytopenic disorder. The presence of anatomic defects, including absent radii (thrombocytopenia-absent radius [TAR] syndrome) or right-heart defects (22q11.2 deletion syndrome), high-tone hearing loss, cataracts before age 50, or interstitial nephritis support the diagnosis of hereditary thrombocytopenia. The blood smear is also essential for identifying patients with potential hereditary thrombocytopenia. For example, large platelets and neutrophil inclusions may indicate the presence of a *MYH9*-related disorder. Upon identification of hereditary thrombocytopenia, proper diagnosis of the specific genetic defect should be pursued whenever possible, as several hereditary thrombocytopenias result in a substantially increased risk of myeloid malignancy, including myelodysplasia and acute myeloid leukemia. Although about 40% of families with inherited thrombocytopenia do not have an identifiable gene defect, this is a rapidly evolving area, and the advent of next-generation sequencing has expanded the phenotypes of some of the classical platelet disorders. Certain reference laboratories are now offering next-generation sequencing panels covering several of the more common hereditary thrombocytopenias, facilitating more specific identification of the causative genetic mutation.

Thrombocytopenia with large platelets (macrothrombocytopenia)

Many inherited thrombocytopenias involve defects in platelet production, whereas megakaryocytopoiesis is largely normal. The platelet mass is distributed to fewer platelets, which results in macrothrombocytopenia. Most inherited thrombocytopenias result in macrothrombocytopenia. Automated particle counters often underestimate the platelet number by counting the large platelets as red cells or leukocytes. Although platelet size may be increased in ITP or myeloproliferative neoplasms (MPNs), the platelet population is typically heterogenous with large- and normal-sized platelets. Any blood smear showing >60% large platelets is highly suspicious for a hereditary macrothrombocytopenia.

The most common of the macrothrombocytopenias, *MYH9*-related thrombocytopenia, is an autosomal dominant macrothrombocytopenia that formerly consisted of the May-Hegglin, Fechtner, Sebastian, and Epstein syndromes. All of these are caused by variants in the *MYH9* gene, which codes for nonmuscle myosin IIA. In addition to macrothrombocytopenia, the peripheral blood film typically demonstrates Döhle body–like inclusions in neutrophils (which are best detected by immunofluorescence, Figure 11-6). Associated clinical features including hearing loss, cataracts, and renal failure are present in some patients. Bleeding symptoms are mild to moderate because platelet function is nearly normal, apart from a reduction in platelet cytoskeleton contraction with resulting reduced clot stability. About 30% of patients have a de novo mutation and therefore a negative family history. Large platelets are also found in a subgroup of VWD type IIB (Montreal platelet disorder) and in both monoallelic and biallelic Bernard-Soulier syndrome, which is characterized by the decreased expression of the platelet GPIb-IX complex, lack of platelet agglutination with high-dose ristocetin, and bleeding (see the section "Disorders of platelet function"). Furthermore, variants in the platelet cytoskeleton proteins beta tubulin (TUBB1), filamin (FLNA), alpha actinin (ACTN1), tropomyosin 4 (TPM4), and DIAPH1, a member of the formin family that regulates microtubule

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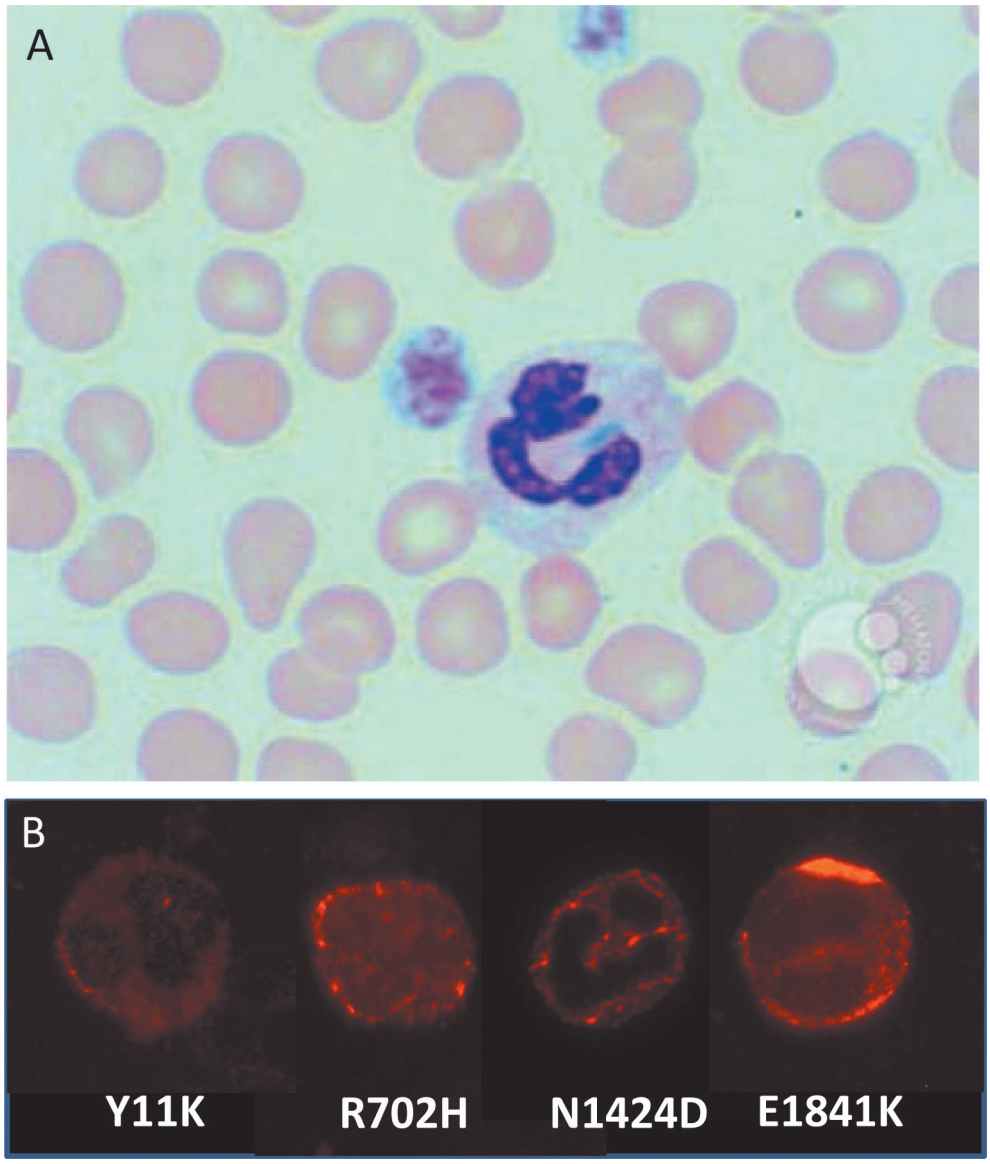

Figure 11-6 ***MYH9*-associated macrothrombocytopenia.** Heterozygous mutations in the *MYH9* gene encoding nonmuscular myosin IIa are the most frequent cause of hereditary macrothrombocytopenia. The mutated protein clusters in the cytoplasm of neutrophils. The inclusion bodies differ in size and shape depending on the mutation. Large inclusion bodies result from mutations in the downstream part of the gene and are visible by both light microscopy and immunofluorescence. More upstream mutations result in smaller inclusion bodies that are visible only with immunofluorescence. (A) Peripheral blood film from a patient with a downstream mutation. A giant platelet is visible. Adjacent to the platelet is a neutrophil containing a large blue Döhle-like body. Source: ASH Image Bank/Julie Braza. (B) Immunofluorescence stain from patients with 4 different mutations.

assembly, result in macrothrombocytopenia. DIAPH1 variants are also associated with sensorineural hearing loss. More severe bleeding disorders associated with macrothrombocytopenia are seen in biallelic BSS, *PRKACG*-related thrombocytopenia, and in patients with activating variants in *ITGA2B/ITGB3*, which cause Glanzmann thrombasthenia.

Hereditary macrothrombocytopenias also occur in association with mutations in specific transcription factors that regulate megakaryocyte and platelet production,

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including *GATA1* (X-linked inheritance and dyserythropoiesis) (see "Disorders of platelet function" for more information). Patients with the Paris-Trousseau/Jacobsen syndrome, an autosomal dominant macrothrombocytopenia, have psychomotor retardation and facial and cardiac abnormalities. This syndrome arises because of deletion of a portion of chromosome 11, 11q23–24, that encompasses the gene encoding the transcription factor friend leukemia integration 1 (FLI1). Autosomal recessive inheritance of variants in this gene alone reproduce the Paris-Trousseau platelet phenotype without the associated cardiac and developmental abnormalities. Gray platelet syndrome (deficiency of alpha granules) results from variants in the *NBEAL2* gene (recessive trait) and generally causes a macrothrombocytopenia. Variants in GFI1b have been shown to cause a platelet defect that is similar to gray platelet syndrome, with loss of platelet granules and variable alterations in platelet function inherited in an autosomal dominant fashion. Activating mutations in *SRC* cause a juvenile myelofibrosis-associated thrombocytopenia inherited in an autosomal dominant fashion.

Thrombocytopenia with normal-sized platelets

Normal-sized platelets are found in 3 autosomal dominant, inherited thrombocytopenias with associated increased risk of myeloid malignancy: *RUNX1* (with variable platelet dysfunction and therefore variable bleeding), *ANKRD26* (mild to no bleeding), and *ETV6* (mild to no bleeding). The risk of malignancy with these disorders is markedly increased. With *RUNX1* defects, the thrombocytopenia is not 100% penetrant. Therefore, genetic screening should include even those family members with normal platelet counts. *RUNX1* variants are also discussed in the section "Disorders of platelet function." The inherited thrombocytopenias associated with increased risk of bone marrow failure are also generally associated with normal platelet size: congenital amegakaryocytic thrombocytopenia (CAMT), TAR, and radioulnar synostosis with amegakaryocytic thrombocytopenia (RUSAT). CAMT, a recessive disorder due to mutations in the c-Mpl receptor, is characterized by severe thrombocytopenia, absence of megakaryocytes in the bone marrow, and a risk of trilineage failure. TAR syndrome is inherited in a compound fashion, with most patients coinheriting a microdeletion of 1q21 encompassing the *RBM8A* gene and 1 of 2 polymorphisms on the other chromosome in *RBM8A* associated with decreased expression. RUSAT results from autosomal dominant inheritance of *HOXA11* variants or autosomal recessive variants in *MECOM*. The autosomal recessive form is associated with an increased risk of bone marrow failure and myelodysplastic syndrome.

Small platelets are typical of Wiskott-Aldrich syndrome (WAS), an X-linked disorder characterized by severe immunodeficiency, small platelets, and eczema (this is described in more detail in the section "Disorders of platelet function"). Two additional autosomal recessive disorders with small platelets have been recently described: *FYB*-related thrombocytopenia with isolated small platelets and thrombocytopenia and a very rare disorder of inflammation, eosinophilia, and microthrombocytopenia due to variants in *ARPC1*.

Establishing the diagnosis of hereditary thrombocytopenia may be difficult. Historically, demonstration of decreased expression of platelet GPIb-IX using flow cytometry has been used to diagnose BSS. Clustering of myosin in granulocytes using an immunofluorescent antibody against nonmuscle myosin heavy chain type IIa may aid in screening for *MYH9*-related disorders. Improvements in sequencing technologies have allowed for the expansion of genetic analyses for BSS, *MYH9*-related thrombocytopenia, CAMT, *GATA1*-related thrombocytopenia, TAR syndrome, and WAS-associated thrombocytopenia. Several laboratories in the United States and Europe now provide these services (see http://www.genetests.org).

KEY POINTS

- Failure to respond to standard ITP therapy (corticosteroids and IVIG) should prompt consideration of a hereditary thrombocytopenia.
- Genetic diagnosis of hereditary thrombocytopenia should be obtained when possible as it will guide additional therapy, genetic counseling, and assessment of cancer risk.

Disorders of platelet function

Disorders of platelet function (see video in online edition) are characterized by variable mucocutaneous bleeding manifestations and excessive hemorrhage following surgical procedures or trauma. Spontaneous hemarthrosis and deep hematomas are unusual in patients with platelet defects. Most patients have mild-to-moderate bleeding manifestations. Platelet aggregation and secretion studies provide evidence for the defect but generally are not predictive of the severity of clinical manifestations. Defects in platelet function may be inherited or acquired, with the latter being far more commonly encountered.

Inherited disorders of platelet function

Table 11-6 provides a classification of inherited disorders associated with impaired platelet function (Figure 11-7).

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Table 11-6 Inherited disorders of platelet function

1. Defects in platelet–vessel wall interaction (disorders of adhesion) a. von Willebrand disease (deficiency or defect in plasma VWF) b. Bernard-Soulier syndrome (deficiency or defect in GPIb) c. GPVI deficiency
2. Defects in platelet-platelet interaction (disorders of aggregation) a. Congenital afibrinogenemia (deficiency of plasma fibrinogen) b. Glanzmann thrombasthenia (deficiency or defect in GPIIb-IIIa) B1: *FERMT3* variants causing LAD-III (GT-like platelet defect with immunodeficiency, poor wound healing, +/- osteopetrosis) B2: *RASGRP2* variants causing deficiency of GalDAG-GEFI and abnormal signaling/activation of GPIIb/IIIa and GPS-like platelet defect
3. Disorders of granule biogenesis a. δ-SPD (isolated, HPS, CHS, Griscelli) b. α-SPD (GPS, ARC, QPS)
4. Disorders of platelet secretion and signal transduction a. Receptor defects (TXA2R, P2Y12, F2RL3, P2RX1, GP6) b. G-protein (Gαq, Gαs, Gαi) abnormalities c. Defects in phosphatidylinositol metabolism and protein phosphorylation • Phospholipase C-β2 deficiency • PKC-θ deficiency d. Abnormalities in arachidonic acid pathways and thromboxane A_2 synthesis • Phospholipase A_2 deficiency • Cyclooxygenase deficiency • Thromboxane synthase deficiency e. Defects in signal transduction • *RASGRP2* (CalDAG-GEF1) • *FERMT3* (kindlin-3) • *PRKACG*
5. Disorders of platelet coagulant-protein interaction (Scott syndrome) Stormorken/York platelet syndrome (increased baseline PS, abnormal calcium flux)
6. Defects related to cytoskeletal/structural proteins a. Wiskott-Aldrich syndrome b. Filamin deficiency (*FLNA*) (*TUBB1*, *ACTN1*, *MYH9* not typically associated with significant platelet dysfunction)
7. Abnormalities of transcription factors leading to functional defects a. *RUNX1* b. *GATA1* c. *FLI1* (Paris-Trousseau platelets, abnormal function) d. *GFI1B*

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CHS, Chediak Higashi Syndrome; GATA1, sex-linked inheritance; GPS, grey platelet syndrome; QPS, Quebec Platelet Syndrome; RUNX1, autosomal dominant.

Of note, not all of these disorders are due to a defect in the platelet per se. Some, such as VWD and afibrinogenemia, result from deficiencies of plasma proteins essential for platelet adhesion or aggregation and are covered elsewhere. Some of these disorders are distinctly rare but shed light on platelet physiology. In the majority of patients with inherited abnormalities of platelet function, the molecular defect remains unknown, suggesting that some of these disorders may be the result of coinheritance of multiple hypofunctional variants. In patients with defects in platelet–vessel wall interactions (adhesion disorders), adhesion of platelets to subendothelium is abnormal. Binding of fibrinogen to the GPIIb-IIIa complex is a prerequisite for platelet aggregation. Disorders characterized by abnormal platelet-platelet interactions (aggregation disorders) arise because of a severe deficiency of plasma fibrinogen (congenital afibrinogenemia) or because of a quantitative or qualitative abnormality of the platelet membrane GPIIb-IIIa complex, which binds fibrinogen (Glanzmann thrombasthenia). The remainder of the platelet function defects are grouped according to the mechanism by which platelet dysfunction occurs, but many of these disorders have not yet been fully molecularly characterized. Those that have are often associated with other clinical manifestations due to effects of the variants on pathways outside the platelet. Patients with defects in platelet secretion and signal transduction are a heterogeneous group lumped together for convenience of

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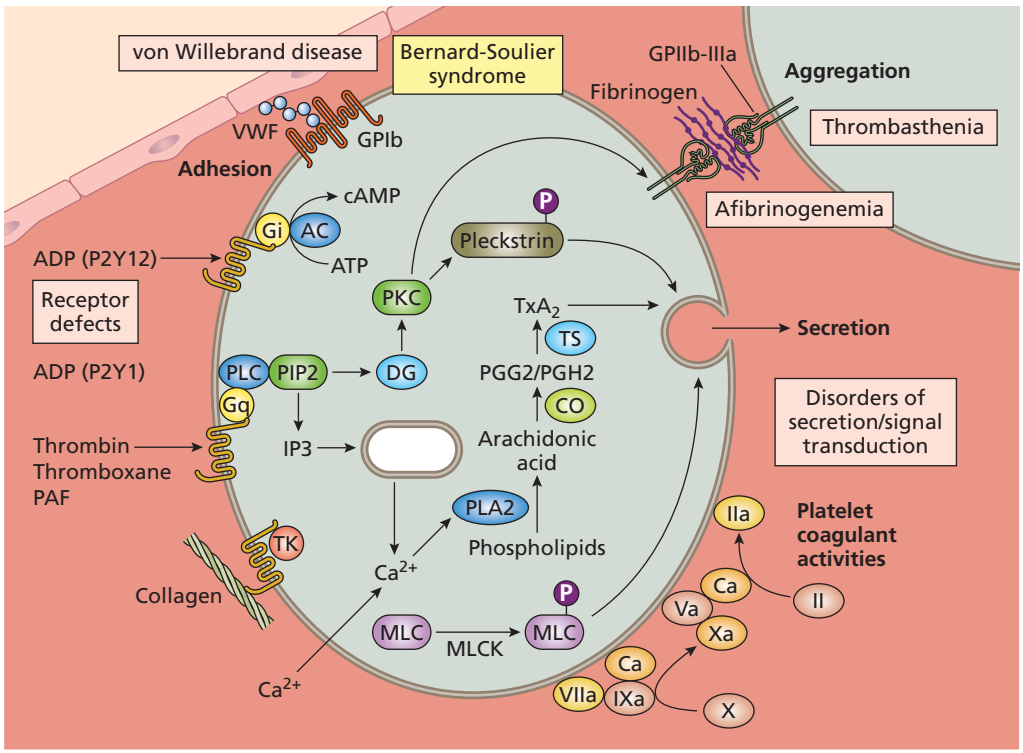

Figure 11-7 Schematic representation of selected platelet responses to activation and inherited disorders of platelet function. Roman numerals in circles represent coagulation factors. AC, adenylyl cyclase; CO, cyclooxygenase; G, guanosine triphosphate–binding protein; IP3, inositol trisphosphate; MLC, myosin light chain; MLCK, myosin light chain kinase; PAF, platelet-activating factor; PIP2, phosphatidylinositol bisphosphate; PLA2, phospholipase A_2; PLC, phospholipase C; TK, tyrosine kinase; TS, thromboxane synthase. Adapted from Rao AK, *Am J Med Sci*. 1998;316:69-76 (© 1998), with permission from the Southern Society for Clinical Investigation.

classification rather than an understanding of the specific underlying abnormality. The major common characteristics in these patients, as currently perceived, are abnormal aggregation responses and an inability to release intracellular granule (dense) contents upon activation of platelet-rich plasma with agonists such as ADP, epinephrine, and collagen. In aggregation studies, the second wave of aggregation is blunted or absent.

Platelet secretion defects result in platelet dysfunction from a variety of mechanisms. Defects related to platelet cytoskeletal or structural proteins also may be associated with platelet dysfunction, often on the basis of impaired signal transduction or platelet granule secretion because of abnormal interactions between cytoskeleton and membrane. Finally, variants in transcription factors (eg, RUNX1, GATA1, FLI1, and GFI1b) that regulate the expression of important platelet proteins may also variably impact platelet function because of abnormal granule packaging. The prevalence and relative frequencies of the various platelet abnormalities remain unknown.

Disorders of platelet adhesion

Bernard-Soulier syndrome

BSS, a rare autosomal recessive platelet function disorder, results from an abnormality in the platelet GPIb-IX complex, which mediates the binding of VWF to platelets and thus plays a major role in platelet adhesion to the subendothelium, especially at high shear rates. GPIb exists in platelets as a complex consisting of GPIb, GPIX, and GPV. There are approximately 25,000 copies of GPIb-IX on the surface of an individual platelet, and these are reduced or abnormal in BSS. Although GPV also is decreased in BSS platelets, it is not required for platelet surface GPIb-IX expression. The platelet count is moderately decreased, and platelets are markedly increased in size on the peripheral film. In platelet aggregation studies, responses to ADP, epinephrine, thrombin, and collagen are normal, and response to ristocetin is decreased or absent, a feature shared with severe VWD. This is because ristocetin-induced platelet agglutination is mediated by binding of VWF to the GPIb

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complex. Unlike in VWD, however, plasma VWF and factor VIII are normal in BSS, and the addition of normal donor VWF does not restore ristocetin-induced agglutination of platelets because of GPIb deficiency on the patient's platelets. Dense granule secretion on activation with thrombin may be decreased in these patients. The diagnosis of BSS is established by demonstrating decreased platelet surface GPIb, which can be performed using flow cytometry. The most severe phenotype is associated with the biallelic form of BSS in which variants are inherited from both parents, resulting in markedly reduced expression and/or function of the GPIb-IX complex. Monoallelic forms (autosomal dominant BSS, benign Mediterranean macrothrombocytopenia, and Bolzano variant of BSS) have been described as well, with a less severe phenotype and decreased expression of GPIb-IX and variable response to ristocetin on platelet function testing.

von Willebrand disease

See "von Willebrand disease" in Chapter 10.

Disorders of platelet aggregation

Glanzmann thrombasthenia

Glanzmann thrombasthenia is a rare autosomal recessive disorder characterized by markedly impaired platelet aggregation and relatively severe mucocutaneous bleeding manifestations compared with most other platelet function disorders. It has been reported in clusters in populations in which consanguinity is common. Normal resting platelets possess approximately 50,000 to 80,000 GPIIb-IIIa complexes on their surface. The primary abnormality in Glanzmann thrombasthenia is a quantitative or qualitative defect in the GPIIb-IIIa complex, a heterodimer consisting of GPIIb and GPIIIa whose synthesis is governed by distinct genes located on chromosome 17. Thus, thrombasthenia may arise because of a mutation in either gene, with decreased platelet expression of the complex. Platelet aggregation is mediated through interaction of GPIIb-IIIa and fibrinogen. In Glanzmann thrombasthenia, platelet aggregation is impaired. Clot retraction, a function of the interaction of GPIIb-IIIa with the platelet cytoskeleton, is also compromised.

Binding of fibrinogen to the GPIIb-IIIa complex on platelet activation is required for aggregation in response to all physiologic agonists. Thus, the diagnostic hallmark of thrombasthenia is the absence or marked decrease of platelet aggregation in response to all platelet agonists (except ristocetin), with absence of both the primary and secondary wave of aggregation. The shape change response is preserved. Platelet-dense granule secretion may be decreased with weak agonists (eg, ADP) but is normal on activation with thrombin. Heterozygotes have approximately half the number of platelet GPIIb-IIIa complexes, but platelet aggregation responses are normal. Although congenital afibrinogenemia is also characterized by absence of platelet aggregation, it can be distinguished from thrombasthenia by a prolonged PT, aPTT, and thrombin time and absent fibrinogen. The diagnosis of thrombasthenia can be confirmed by demonstrating decreased platelet expression of the GPIIb-IIIa complex using flow cytometry. A subgroup of disorders of the GPIIb-IIIa complex are inherited dominantly. These patients have moderately decreased platelet numbers and large platelets. The underlying cause is a mutation in the transmembrane or intracellular part of the integrin, which results in permanent activation of the GPIIb-IIIa complex. Finally, recently described defects in genes downstream of the integrin complex in *FERMT3* and *RASGRP2* result in Glanzmann-like platelet function defects with severe bleeding phenotype and markedly abnormal platelet responses by light transmission aggregometry but only mild or moderate decrease in expression of GPIIb-IIIa on the surface of platelets.

Disorders of platelet secretion and signal transduction

As a unifying theme, patients lumped into this remarkably heterogeneous group generally are characterized by impaired dense granule secretion and the absence of a second wave of aggregation upon stimulation of platelet-rich plasma with ADP or epinephrine; responses to collagen, thromboxane analog (U46619), and arachidonic acid also may be impaired. Conceptually, platelet function is abnormal in these patients, either because the granule contents are diminished (storage pool deficiency) or because the mechanisms mediating or potentiating aggregation and secretion are impaired (Table 11-6). Identification of the underlying defect is often very difficult, and in many patients, it is caused by a combination of several minor abnormalities.

Defects of granule biogenesis

Many of the defects of granule biogenesis result in a common phenotype called storage pool deficiency (SPD). SPD refers to deficiency in platelet content of dense granules (δ-SPD), α-granules (δ-SPD), or both types of granules (αδ-SPD). Often, the platelet phenotype in these disorders is part of a broader syndromic disease and can be recognized by the other systemic manifestations.

Patients with δ-SPD have a mild-to-moderate bleeding diathesis. In platelet studies, the second wave of aggregation in response to ADP and epinephrine is absent or

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blunted, and the collagen response is markedly impaired. Normal platelets possess 3 to 8 dense granules (each 200 to 300 nm in diameter). Under the electron microscope, dense granules are decreased in δ-SPD platelets. By direct biochemical measurements, the total platelet and granule ATP and ADP contents are decreased along with other dense granule constituents, including calcium, pyrophosphate, and serotonin.

δ-SPD has been reported in association with other inherited disorders, such as Hermansky-Pudlak syndrome (HPS) (oculocutaneous albinism and increased reticuloendothelial ceroid), Chediak-Higashi syndrome, and Griscelli syndrome. Some patients with WAS have been reported to manifest δ-SPD as part of their platelet phenotype. The simultaneous occurrence of δ-SPD and defects in skin pigment granules, as in HPS, point to commonalities in the pathways that govern synthesis and trafficking of platelet-dense granules and melanosomes.

In northwest Puerto Rico, HPS affects 1 of every 1800 individuals. There are at least 9 known HPS-causing genes, with most patients having HPS-1 and being from Puerto Rico. HPS is autosomal recessive, and heterozygotes classically have no clinical findings. In addition to albinism, many patients have congenital nystagmus and decreased visual acuity. Two additional manifestations associated with certain HPS subtypes are granulomatous colitis and pulmonary fibrosis. With next-generation sequencing, the phenotype for some HPS variants is expanding and now also includes neutropenia and immunodeficiency.

Chediak-Higashi syndrome is a rare autosomal recessive disorder characterized by δ-SPD, oculocutaneous albinism, immune deficiency, cytotoxic T and natural killer cell dysfunction, neurologic symptoms, and the presence of giant cytoplasmic inclusions in different cells. It arises from mutations in the lysosomal trafficking regulator (*LYST*) gene on chromosome 1.

Patients with gray platelet syndrome have an isolated deficiency of platelet α-granule contents. The name refers to the initial observation that the platelets have a gray appearance with a paucity of granules on the peripheral blood film. These patients have a bleeding diathesis, mild thrombocytopenia, and a prolonged bleeding time. The inheritance pattern is variable; autosomal recessive, autosomal dominant, and sex-linked patterns have been described. Classical GPS (autosomal recessive) appears to be due to variants in the *NBEAL2* gene. The autosomal dominant form is associated with variants in *GFI1b* and red cell anisocytosis, whereas the X-linked form has been associated with variants in *GATA1*. Finally, in patients with arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome, caused by variants in *VPS33B* or *VIPAS39*, there is low α-granule content and platelet dysfunction in a setting of fairly severe systemic disease that is often lethal in early childhood. In all of these disorders, under the electron microscope, platelets and megakaryocytes reveal absent or markedly decreased α-granules. The platelets are severely and selectively deficient in α-granule proteins, including PF4, βTG, VWF, thrombospondin, fibronectin, factor V, and platelet-derived growth factor. In some patients, plasma PF4 and βTG are raised, suggesting that the defect is not in their synthesis by megakaryocytes but rather in their packaging into granules. Platelet aggregation responses are variable. Responses to ADP and epinephrine are normal in most patients; in some patients, aggregation responses to thrombin, collagen, and ADP are impaired.

The Quebec platelet disorder is an autosomal dominant disorder associated with delayed bleeding and abnormal proteolysis of α-granule proteins (including fibrinogen, factor V, VWF, thrombospondin, multimerin, and P-selectin) resulting from increased amounts of platelet urokinase-type plasminogen activator (uPA). Patients have normal to reduced platelet counts, proteolytic degradation of α-granule proteins, and a defective aggregation response with epinephrine. This disorder is caused by tandem duplication of a 78-kb region of chromosome 10 containing *PLAU* (the uPA gene), a mechanism unique among inherited platelet disorders.

Defects in platelet signal transduction and platelet activation

Signal transduction mechanisms encompass processes that are initiated by the interaction of agonists with specific platelet receptors and include responses such as G-protein activation and activation of phospholipase C and phospholipase A_2 (Figure 11-7). Patients with disorders of platelet signal transduction and activation present with variable bleeding ranging from mild platelet-type bleeding (similar to that seen in patients treated with antiplatelet therapies) to more severe bleeding, depending on the degree of impairment of platelet function.

Patients with receptor defects have impaired responses because the platelet surface receptors for a specific agonist are decreased. Such defects have been documented in the P2Y12 ADP receptor (the receptor targeted by thienopyridines such as clopidogrel), TxA_2 receptor, collagen receptors (GPIa-IIa and GPVI), and epinephrine receptor. Because ADP and TxA_2 play a synergistic role in platelet responses to several agonists, patients with defects in the receptors for these agonists manifest abnormal aggregation responses to multiple agonists.

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G proteins serve as a link between surface receptors and intracellular effector enzymes and defects in G protein (Gαq, Gαi, and Gαs) activation can impair platelet signal transduction. As G proteins are required in many cell types, affected patients typically have syndromic phenotypes, often associated with neural deficiencies.

Downstream abnormalities in platelet function have also been identified, such as defects in phospholipase C activation (phospholipase C-β2 and PKC-θ), calcium mobilization, and pleckstrin phosphorylation. A major platelet response to activation is liberation of arachidonic acid from phospholipids and its subsequent oxygenation to TxA_2, which plays a synergistic role in the response to several agonists. Patients have been described with impaired thromboxane synthesis because of congenital deficiencies of phospholipase A_2, cyclooxygenase, and thromboxane synthase.

Disorders of platelet procoagulant activities

Platelets play a major role in blood coagulation by providing the surface on which several specific key enzymatic reactions occur. In resting platelets, there is an asymmetry in the distribution of some of the phospholipids such that phosphatidylserine and phosphatidylethanolamine are located predominantly on the inner leaflet, whereas phosphatidylcholine has the opposite distribution. Platelet activation results in a redistribution with expression of phosphatidylserine on the outer surface, mediated by phospholipid scramblase. The exposure of phosphatidylserine on the outer surface is an important event in the expression of platelet procoagulant activities. Rare patients have been described in whom this process is impaired, and this is referred to as Scott syndrome. In these patients, who have a bleeding disorder, the bleeding time and platelet aggregation responses are normal. Recently, a second group of platelet disorders has been associated with abnormally increased expression of phosphatidylserine at baseline and increased intracellular platelet Ca^{2+} levels due to abnormal function of the Ca^{2+} release-activated Ca^{2+} channels formed by a pore protein (ORAI1) and Ca^{2+} sensor STIM1. Variants in either *ORAI1* or *STIM1* (activating mutations) have been associated with Stormorken syndrome/York platelet syndrome, a group of disorders characterized by bleeding, abnormal platelets, and myopathy.

Other abnormalities

Platelet function abnormalities have been described in association with other entities such as WAS, GATA1, and FLI1. More recently, abnormal platelet function has been documented in patients with mutations in transcription factor RUNX1, which results in dysregulation of platelet biogenesis in general and abnormal expression of multiple platelet genes including *GATA1*, *MYH9*, *NFE2*, *MYL9*, and *PKC*. Patients with RUNX1 mutations have hereditary thrombocytopenia, platelet dysfunction, and predisposition to acute leukemia.

Treatment of inherited platelet function defects

Because of the wide disparity in bleeding manifestations, management needs to be individualized. Platelet transfusions are indicated in the management of significant bleeding and in preparation for surgical procedures. For severe bleeding, especially in patients with Bernard-Soulier syndrome and Glanzmann thrombasthenia, platelet transfusion should be administered when clinically necessary to provide normally functioning platelets. The general risks associated with platelet transfusion, common to all patients, include the risk of transfusion reactions and potential transmission of infectious agents. In those patients with the most severe bleeding risk, there is also the increased risk of alloimmunization due to the lack of key platelet GPs. For example, patients with Glanzmann thrombasthenia (more commonly) and BSS (less often) may develop alloantibodies against GPIIb-IIIa and GPIb, respectively, which compromise the efficacy of subsequent platelet transfusions. This can result in refractoriness to platelet transfusion and, in women of childbearing age, can also cause devastating neonatal/fetal thrombocytopenia. Therefore, efforts should be made to minimize platelet transfusion in those patients at risk of alloimmunization. These efforts include early treatment of bleeding episodes to avoid platelet transfusion, and early use of platelet transfusions in severe bleeding episodes in order to minimize the total amount of platelets needed, since functional platelets are an important component of the coagulation cascade. Adjunctive therapies are important for the treatment of all patients with platelet disorders.

Desmopressin (DDAVP) is a synthetic analogue of the antidiuretic hormone vasopressin and exerts its procoagulant effect by increasing the circulating levels of factor VIII and VWF. While desmopressin can improve platelet function in congenital platelet disorders, uremia, and during cardiopulmonary bypass, the specific mechanism of action is not clear. Desmopressin may be administered intravenously, subcutaneously, or intranasally. Documentation of a shortened bleeding time, or improved platelet function is not required for use. Intranasal desmopressin is indicated for heavy menstrual bleeding in the setting of platelet disorders, mucosal bleeding such as epistaxis, minor surgical procedures, or other minor bleeding. Tachyphylaxis occurs. Fluid restriction after administration is important

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to prevent hyponatremia as an important side effect is decreased urine output for 18-24 hours after administration. Patients also report flushing, headache, and may have tachycardia.

Recombinant factor VIIa has been approved for the management of bleeding events in patients with Glanzmann thrombasthenia and has been used in some other inherited defects. Although rFVIIa has been shown to be effective for the management of severe bleeding in patients with platelet function defects, rFVIIa is costly and may be associated with adverse (thrombotic) events. Use of rFVIIa is important in severe bleeding episodes or for surgical procedures in combination with transfusion to minimize total transfusions and quickly control bleeding in patients with severe platelet disorders.

Estrogen in combination with progestins, as in oral contraceptive agents, is useful for home management of heavy menstrual bleeding in patients with bleeding disorders, including platelet function disorders and VWD. Additionally, use of long acting regional contraceptives (LARCs, such as the progesterone coated intrauterine device) has gained significant support in the literature and has shown excellent safety and tolerability in women with platelet disorders. These agents can be used in combination with antifibrinolytics in carefully selected patients for maximal control of heavy menstrual bleeding and allow for significant improvement in quality of life and symptom control.

Antifibrinolytic agents (epsilon-aminocaproic acid and tranexamic acid [TXA]) are commonly used adjunctive hemostatic therapies. These agents, which are lysine analogues, inhibit plasmin-mediated thrombolysis and exert their effect through clot stabilization and prevention of early dissolution. Thus, these agents may be effective in prevention of rebleeding, a common problem in individuals with bleeding disorders, especially in areas with increased fibrinolysis, such as the oral cavity, nasal cavity and gastrointestinal tract. These agents may be administered intravenously, orally, or topically in amenable circumstances, and are used either therapeutically for bleeding or prophylactically as part of perioperative management. Treatment of mucosal bleeding commonly includes the use of antifibrinolytic agents in conjunction with desmopressin; this combination is also effective in bleeding from other sites—for example, in the management of heavy menstrual bleeding. Antifibrinolytic agents have been used widely for many years, have a documented safety profile, and are well tolerated in most patients. Commonly reported side effects include headache and abdominal discomfort; however, these symptoms do not preclude its continued use if ameliorated with other agents, such as acetaminophen. Antifibrinolytic agents should be used with caution in patients with a history of thrombosis or atherosclerosis and are contraindicated when hematuria is present because obstructive uropathy secondary to ureteral clots may develop.

Bone marrow transplantation is being used increasingly in the most severe platelet disorders (WAS, Glanzmann thrombasthenia) and successful gene therapy trials suggest this may also be an option in some disorders (eg, WAS).

A basic therapeutic principle in all patients with platelet disorders is to prevent iron-deficiency anemia. Red blood cells are required for sufficient hemostasis. Iron-deficiency anemia is common in this population, especially in women of childbearing age. Oral iron supplementation may be insufficient to normalize iron stores, and intravenous iron therapy may be required.

KEY POINTS

- Patients with inherited platelet defects typically have mucocutaneous bleeding manifestations.
- Patients with BSS have thrombocytopenia, large platelet size, and a defect in the platelet GPIb-V-IX complex, leading to impaired binding of VWF and adhesion.
- Patients with Glanzmann thrombasthenia have absent or decreased platelet GPIIb-IIIa, leading to impaired binding of fibrinogen and absent aggregation to all of the usual agonists except ristocetin.
- Patients with δ-storage pool deficiency have decreased dense granule contents; some patients may have associated albinism, nystagmus, and neurologic manifestations.
- Patients with the gray platelet syndrome have decreased a-granule contents.
- In a substantial number of patients with abnormal aggregation responses, the underlying mechanisms are unknown. Some patients have defects in platelet activation and signaling mechanisms.

Acquired disorders of platelet function

Alterations in platelet function occur in many acquired disorders of diverse etiology (Table 11-7), of which drugs are the most frequent. In most of the non-drug-induced acquired platelet function disorders, the specific biochemical and pathophysiologic aberrations leading to platelet dysfunction are poorly understood. As with inherited disorders of platelet function, in acquired disorders, bleeding is usually mucocutaneous with a wide and unpredictable spectrum of severity. The usual laboratory tests that suggest platelet dysfunction include abnormal results in

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Table 11-7 Disorders in which acquired defects in platelet function are recognized

Uremia
Myeloproliferative neoplasms
Acute leukemias and myelodysplastic syndrome
Dysproteinemias
Cardiopulmonary bypass
Acquired storage pool deficiency
Acquired von Willebrand disease
Antiplatelet antibodies
Drugs and other agents

studies of platelet aggregation or the platelet function analyzer 100 (PFA-100). In patients with acquired platelet dysfunction, the correlation between abnormalities in platelet aggregation studies and clinical bleeding remains weak.

Drugs that inhibit platelet function

Many drugs affect platelet function (see video in online edition). Moreover, the impact of concomitant administration of multiple drugs, each with a mild effect on platelet function, is unknown. Because of their widespread use, aspirin and nonsteroidal anti-inflammatory agents are an important cause of platelet inhibition in clinical practice. Aspirin ingestion results in inhibition of platelet aggregation and secretion upon stimulation with ADP, epinephrine, and low concentrations of collagen. Aspirin irreversibly acetylates and inactivates platelet cyclooxygenase (COX-1), leading to the inhibition of synthesis of endoperoxides (prostaglandin G_2 and H_2) and TxA_2. Typically, it is recommended to wait 7 to 10 days after cessation of aspirin ingestion to perform studies intended to assess platelet function and elective invasive procedures to ensure that the antiplatelet effect is gone. Nonsteroidal anti-inflammatory drugs also impair platelet function by inhibiting the cyclooxygenase enzyme. Compared with aspirin, the inhibition of cyclooxygenase by these agents generally is short-lived and reversible (1 to 2 days). Cyclooxygenase-2 inhibitors (such as celecoxib) do not inhibit platelet aggregation responses.

Ticlopidine, clopidogrel, and prasugrel are orally administered thienopyridine derivatives that inhibit platelet function by irreversibly binding to the platelet P2Y12 receptor at the ADP-binding site. In contrast, ticragrelor is a reversible inhibitor of platelet function that binds to P2Y12 at a site different from ADP and allosterically blocks access of ADP to the receptor. These drugs prolong the bleeding time and inhibit platelet aggregation responses to several agonists, including ADP, collagen, epinephrine, and thrombin, to various extents depending on agonist concentrations. The active drug/metabolites of the irreversible antiplatelet drugs (aspirin, clopidogrel, prasugrel) disappear from the circulation within a relatively short time (aspirin 1 hour; clopidogrel ~5 hours; prasugrel ~8 to 10 hours). However, ticagrelor reaches such high plasma levels that it is present in the circulation for 72 to 96 hours despite its relatively short half-life of 7 to 8 hours. This has major implications for patient management. While hemostasis can be normalized after cessation of the irreversible platelet function inhibitors within a reasonable time by platelet transfusion, this intervention is rather ineffective in the case of ticagrelor and any invasive procedures with increased bleeding risk should be postponed by 96 hours, if possible.

GPIIb-IIIa receptor antagonists are compounds that inhibit platelet fibrinogen binding and platelet aggregation. These include the Fab fragment of a monoclonal antibody against the GPIIb-IIIa receptor (abciximab), a synthetic peptide containing the RGD sequence (eptifibatide), and a peptidomimetic (tirofiban). They are potent inhibitors of aggregation in response to all agonists (except ristocetin) and prolong the bleeding time. DITP (secondary to drug-dependent antibodies) occurs in 0.2% to 1.0% of patients on first exposure to GPIIb-IIIa antagonists (see the section "Drug-induced immune thrombocytopenia").

A host of other medications and agents, including oncologic drugs and food substances (such as fish oils and turmeric) inhibit platelet responses, but the clinical significance for many is unclear. β-lactam antibiotics, including penicillins and cephalosporins, inhibit platelet aggregation responses and may contribute to a bleeding diathesis at high doses. The platelet inhibition appears to be dose dependent, taking approximately 2 to 3 days to manifest and 3 to 10 days to abate after drug discontinuation. The clinical significance of the effect of antibiotics on platelet function remains unclear. The general context in which bleeding events are encountered in patients on antibiotics prevents identification of the precise role played by antimicrobials because of the presence of concomitant factors (eg, thrombocytopenia, DIC, infection, vitamin K deficiency). Avoidance or discontinuation of a specifically indicated antibiotic usually is not necessary.

Evidence is growing that SSRIs inhibit platelet function in vivo. Serotonin in plasma is taken up by platelets, incorporated into dense granules, and secreted upon platelet activation. SSRIs inhibit the uptake of serotonin, platelet aggregation, and secretion responses to activation. In epidemiologic studies, patients on SSRIs have had increased gastrointestinal bleeding and increased bleeding with surgery.

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Given the increasing use of herbal medicines and food supplements, their role and interaction with pharmaceutical drugs need to be considered in the evaluation of patients with unexplained bleeding.

Myeloproliferative neoplasms

Bleeding tendency, thromboembolic complications, and qualitative platelet defects are all recognized in MPNs, which include essential thrombocythemia, polycythemia vera, primary myelofibrosis, and chronic myelogenous leukemia. Platelet function abnormalities result principally from development from an abnormal stem cell clone, but some alterations may be secondary to enhanced platelet activation in vivo. The fact that many of these patients may also be on aspirin may compound the platelet function defect. The clinical impact of in vitro qualitative platelet defects, which are observed even in asymptomatic patients, is unclear.

Numerous studies have examined platelet function and morphology in patients with MPNs. Large platelets may be observed. Under the electron microscope, findings include reduction in dense and α-granules, alterations in the open canalicular and dense tubular systems, and reduced mitochondria. The bleeding time is prolonged in a minority (17%) of MPN patients and does not correlate with an increased risk of bleeding. Platelet aggregation responses are highly variable in MPN patients and often vary in the same patient over time. Decreased platelet responses are more common, although some patients demonstrate enhanced responses to agonists. In one analysis, responses to ADP, collagen, and epinephrine were decreased in 39%, 37%, and 57% of patients, respectively. The impairment in aggregation to epinephrine is more commonly encountered than with other agonists; however, a diminished response to epinephrine is not pathognomonic of an MPN. Platelet abnormalities described in MPN include decreased platelet α_2-adrenergic receptors, TxA_2 production, and dense granule secretion and abnormalities in platelet surface expression of GPIIb-IIIa complexes, GPIb, and GPIa-IIa.

Platelets from patients with polycythemia vera and myelofibrosis, but not essential thrombocythemia or chronic myelogenous leukemia, have been shown to have reduced expression of the TPO receptor (Mpl) and reduced TPO-induced tyrosine phosphorylation of proteins. MPN patients have been reported to have defects in platelet-signaling mechanisms. An acquired decrease in plasma VWF has been documented in some MPN patients with elevated platelet counts and may contribute to the hemostatic defect.

Acute leukemias and myelodysplastic syndromes

The major cause of bleeding in these conditions is thrombocytopenia. In patients with normal or elevated platelet counts, however, bleeding complications may be associated with platelet dysfunction and altered platelet and megakaryocyte morphology, on a background of compromised mucosa from chemotherapy. Acquired platelet defects associated with clinical bleeding are more common in acute myeloid leukemia but also have been reported in acute lymphoblastic leukemia, hairy cell leukemia, and myelodysplastic syndromes.

Dysproteinemias

Excessive clinical bleeding may occur in patients with dysproteinemias, and this appears to be related to multiple mechanisms including platelet dysfunction, specific coagulation factor abnormalities, hyperviscosity, and alterations in blood vessels because of amyloid deposition. Qualitative platelet defects occur in some patients and have been attributed to coating of platelets by the paraprotein. The bleeding tendency may be aggravated by concomitant inhibition of VWF by the paraprotein.

Uremia

Patients with uremia are at an increased risk for bleeding complications. The pathogenesis of the hemostatic defect in uremia remains unclear, but major factors include platelet dysfunction and impaired platelet–vessel wall interactions, comorbid conditions, anemia, and the concomitant use of medications that affect hemostasis. The bleeding time may be prolonged.

Multiple platelet function abnormalities are recognized in uremia, including impaired adhesion, aggregation, and secretion. These hemostatic defects may be linked to the accumulation of dialyzable and nondialyzable molecules in the plasma of patients with renal failure. One such compound, guanidinosuccinic acid, accumulates in plasma, inhibits platelets in vitro, and stimulates generation of nitric oxide, which inhibits platelet responses by increasing levels of cellular cyclic guanosine monophosphate.

Aggressive dialysis ameliorates the uremic bleeding diathesis in most patients. Hemodialysis and peritoneal dialysis are equally effective. Platelet transfusion is indicated in the management of acute major bleeds. Other treatments including desmopressin, cryoprecipitate, and conjugated estrogens have also been shown to be beneficial. Elevation of the hematocrit with packed red blood cells or recombinant erythropoietin may improve platelet adhesion and correct mild bleeding in uremic patients. The beneficial effect of red blood cells has been attributed to rheologic factors whereby the red blood cells exert an outward radial pressure promoting platelet–vessel wall interactions. Other factors predisposing to bleeding in patients with renal failure include concomitant administration of antiplatelet agents or anticoagulant medications.

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Acquired SPD

Several patients have been reported in whom dense granule SPD appears to be acquired. This defect probably reflects the release of dense granule contents because of in vivo platelet activation or production of abnormal platelets. Acquired SPD has been observed in patients with antiplatelet antibodies, systemic lupus erythematosus, chronic ITP, DIC, HUS, renal transplant rejection, multiple congenital cavernous hemangiomas, MPN, acute and chronic leukemias, severe valvular disease, and in patients undergoing cardiopulmonary bypass.

Acquired von Willebrand syndrome

Acquired von Willebrand syndrome is described in detail in Chapter 10.

Antiplatelet antibodies and platelet function

Binding of antibodies to platelets may produce several effects—including accelerated destruction, platelet activation, cell lysis, aggregation, secretion of granule contents, and outward exposure of phosphatidylserine. In ITP, antibodies are directed against specific platelet surface membrane GPs that play a major role in normal platelet function, including GPIb, GPIIb-IIIa, GPIa-IIa, GPVI, and glycosphingolipids. Patients with ITP are generally assumed to have normal or supranormal platelet function. However, some may have impaired platelet function due to antiplatelet antibodies that interfere with platelet function. Other patients may have autoantibodies that impair platelet function but do not induce accelerated platelet clearance or thrombocytopenia.

KEY POINTS

- Alterations in platelet function are described in many acquired disorders of diverse etiologies; the clinical significance remains unclear in many cases.
- A careful drug history should be taken in any patient suspected to have platelet dysfunction.
- Aspirin, nonsteroidal anti-inflammatory agents, and other medications are a major cause of acquired platelet dysfunction.
- Patients with MPNs may have altered platelet function that contributes to bleeding manifestations.
- High platelet counts, as observed in MPN patients, may be associated with a loss of high-molecular-weight multimers of VWF in plasma.
- Patients with renal failure may have impaired platelet function related to accumulation of substances in plasma that inhibit platelet function. Vigorous dialysis is a major part of management of platelet dysfunction in these patients.

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